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## ON THREE SPECIES OF HIPPOBOSCIDAE (DIPTERA) ON BIRDS IN ONTARIO<sup>1</sup>

GORDON F. BENNETT

### Abstract

*Ornithomyia fringillina*, *Ornithoica vicina*, and *Lynchia americana* were collected from 16% of 6448 birds (of 84 species) examined in Algonquin Park, Ontario, during July–September of 1957–60; *fringillina* occurred most frequently. Hippoboscids occurred more commonly and had better survival on immature birds. Sparrows and thrushes harbored *fringillina* more frequently than other birds; certain birds, such as purple finches, were unsuitable hosts. Experimental evidence indicated that female flies, once on a bird, usually remained on it; males had a tendency to wander. Female *fringillina* would return to an individual bird on which they had previously lived. Both *vicina* and *americana* behaved in a manner similar to *fringillina*, but the data on them are not as abundant. Female *fringillina* lived up to 81 days, producing puparia every 5 days; males were shorter-lived. Female *vicina* and *americana* lived for 120–135 days, producing puparia every 5 days. Male *fringillina* required blood daily; females required blood daily to maintain maximum reproduction but survived if fed every 3–4 days. Puparia of *fringillina* pass through diapause, require a minimum of 150 days at 75° F; puparia of *vicina* and *americana* do not pass through diapause and require a shorter period for metamorphosis.

### Introduction

Although hippoboscids, particularly *Ornithomyia fringillina* (Curtis), *Ornithoica vicina* (Walker), and *Lynchia americana* (Leach), are frequently collected (3, 4), knowledge of their life history and behavior is limited. During the summers of 1957–1960 many birds were examined in Algonquin Park, Canada; the hippoboscids on them were removed and some were maintained on captive birds. *Ornithomyia fringillina* was taken most frequently and most of the studies were carried out on it. The incidence of these flies is reported, together with some aspects of their behavior as determined from field observations and laboratory studies. Other observations on the flies include those on longevity, frequency of feeding, rate of puparial production, suitability of different birds as breeding hosts, and mating. The rates of metamorphosis under different conditions of temperature were studied.

### Materials and Methods

Field studies were carried out at the Wildlife Research Station, Ontario Department of Lands and Forests, Lake Sasajewan, Algonquin Park, Canada.

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Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto, Canada. This work was made possible by a research grant to the Ontario Research Foundation by the Department of Commerce and Development, Province of Ontario.

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Birds were caught in Japanese mist nets and baited funnel traps. A greater variety of birds were taken by the former method, but the latter caught more fringillids. A bird, entangled in a mist net, was placed, together with the hand holding the bird, as quickly as possible into a polyethylene bag (18 in.  $\times$  12 in.) of 1/2 mil thickness, which was then closed tightly about the wrist with the other hand. The feathers of the bird were ruffled with the hand holding the bird to disturb the flies. Flies usually left the bird in less than 3 minutes but an additional minute was allowed to make certain all had been removed. The flies were then trapped in a corner of the bag while the bird was removed. Handling or release of a bird from the net before it was placed in a bag was avoided to prevent loss of flies. Birds in traps were caught as quickly as possible and the flies were removed as described. These methods proved highly satisfactory as neither the flies nor the bird were injured. Later handling of the flies was facilitated by use of an aspirator and (or) carbon dioxide anaesthesia.

Captured flies were maintained on captive birds; white-throated sparrows, slate-colored junco, song sparrow, white-winged crossbill, and saw-whet owl were especially useful for this purpose. These birds were confined in a "cage within a cage" (Fig. 1). The inner cage (8 in.  $\times$  8 in.  $\times$  8 in.) with one open side was constructed of 3/8-in. or 1/2-in. hardware cloth. It was suspended on two hooks against one wall of the outer cage (12 in.  $\times$  12 in.  $\times$  12 in.). Three

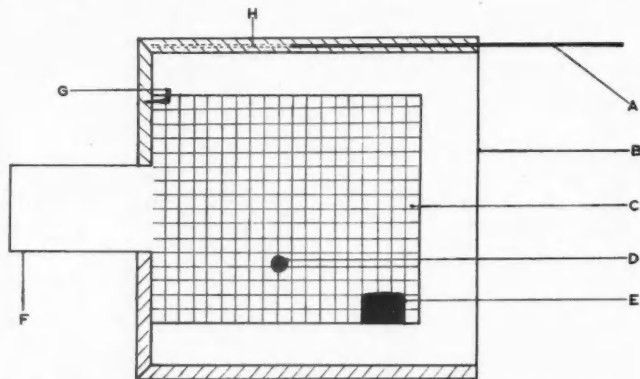


FIG. 1. Diagram of the cage employed in maintaining hippoboscids. A, sliding glass top; B, nylon screen; C, holding cage of 1/2-in. mesh hardware cloth for bird; D, perch; E, food dish; F, cloth sleeve; G, hook to hang cage C on; H, slot for sliding glass panel.

sides and the bottom of the outer cage were made of wood, the fourth of fine nylon mesh, and the top was a sliding glass plate. A sleeve, 4 in. in diameter, on one wall of the outer cage, permitted entry into the inner cage. The inner cage was provided with a perch and food dishes and was serviced through the sleeve. The cages could be cleaned by removing the inner one through the top of the outer one. The double arrangement of cages gave the flies a place where they could not be eaten by the bird, and allowed puparia to drop free of the bird to the floor where they could not be destroyed.

TABLE I

Number of *fringillina* (OF) and *vicina* (OV) on birds captured in Algonquin Park, Ontario  
(July 6-Sept. 20 in 1958-59, August 6-Sept. 13 in 1960)

Bird species	Adults					Immature				
	No. exam.	No. with OF	No. of OF	No. with OV	No. of OV	No. exam.	No. with OF	No. of OF	No. with OV	No. of OV
Tytonidae										
Saw-whet owl										
<i>Aegolius acadica</i>	2	1	3	—	—	1	—	—	—	—
Picidae										
Downy woodpecker										
<i>Dendrocopus pubescens</i>	14	1	2	—	—	15	1	1	—	—
Hairy woodpecker										
<i>Dendrocopus villosus</i>	5	—	—	—	—	5	3	5	—	—
Yellow-bellied sapsucker										
<i>Sphyrapicus varius</i>	4	—	—	—	—	16	2	4	1	1
*Flicker										
<i>Colaptes auratus</i>	13	3	3	—	—	37	7	8	—	—
Tyrannidae										
King bird										
<i>Tyrannus tyrannus</i>	2	—	—	—	—	12	3	4	—	—
Yellow-bellied flycatcher										
<i>Empidonax flaviventris</i>	9	—	—	—	—	15	1	1	—	—
Empidonax flycatchers										
<i>Empidonax minimus, traillii</i>	21	2	2	—	—	107	6	6	—	—
Wood Pewee										
<i>Contopus virens</i>	3	—	—	—	—	7	—	—	1	1
Corvidae										
Blue jay										
<i>Cyanocitta cristata</i>	16	3	4	2	2	18	5	7	5	8
Canada jay										
<i>Perisoreus canadensis</i>	13	—	—	3	3	11	2	2	4	20
Paridae, Troglodytidae										
Black-capped chickadee										
<i>Parus atricapillus</i>	47	1	1	2	2	118	7	7	4	5
Hudsonian chickadee										
<i>Parus hudsonicus</i>	14	—	—	—	—	22	1	1	2	3
Winter wren										
<i>Troglodytes troglodytes</i>	1	1	2	1	1	5	—	—	—	—
Turdidae										
Robin										
<i>Turdus migratorius</i>	25	5	13	1	1	78	18	40	6	22
*Wood thrush										
<i>Hylocichla mustelina</i>	—	—	—	—	—	3	2	5	—	—
*Hermit thrush										
<i>Hylocichla guttata</i>	12	1	1	—	—	34	11	16	—	—
*Olive-backed thrush										
<i>Hylocichla ustulata</i>	66	2	2	—	—	133	49	103	7	10
*Gray-cheeked thrush										
<i>Hylocichla minima</i>	11	2	3	—	—	43	13	27	1	1
*Veery										
<i>Hylocichla fuscescens</i>	15	1	3	—	—	28	10	13	1	1
Sylviidae										
Golden-crowned kinglet										
<i>Regulus satrapa</i>	25	1	2	2	4	35	—	—	4	8
Ruby-crowned kinglet										
<i>Regulus calendula</i>	2	—	—	—	—	18	2	2	4	7
Bombycillidae, Sturnidae										
Cedar waxwing										
<i>Bombycilla cedrorum</i>	11	3	4	—	—	30	9	13	1	1
Starling										
<i>Sturnus vulgaris</i>	1	—	—	—	—	4	2	2	—	—
Vireonidae										
Solitary vireo										
<i>Vireo solitarius</i>	6	1	1	—	—	23	—	—	—	—
Red-eyed vireo										
<i>Vireo olivaceus</i>	31	1	1	—	—	81	6	10	—	—
Philadelphia vireo										
<i>Vireo philadelphicus</i>	2	—	—	—	—	19	2	4	—	—

TABLE I (Concluded)

Number of *fringillina* (OF) and *vicina* (OV) on birds captured in Algonquin Park, Ontario  
(July 6–Sept. 20 in 1958–59, August 6–Sept. 13 in 1960)

Bird species	Adults					Immature				
	No. exam.	No. with OF	No. of OF	No. with OV	No. of OV	No. exam.	No. with OF	No. of OF	No. with OV	No. of OV
<b>Parulidae</b>										
*Black and white warbler										
<i>Mniotilta varia</i>	32	1	1	—	—	74	8	11	1	1
Tennessee warbler										
<i>Vermivora peregrina</i>	18	—	—	—	—	174	1	1	—	—
*Nashville warbler										
<i>Vermivora ruficapilla</i>	139	7	10	—	—	484	25	35	—	—
*Magnolia warbler										
<i>Dendroica magnolia</i>	57	4	4	—	—	188	18	22	—	—
Cape May warbler										
<i>Dendroica tigrina</i>	2	—	—	—	—	27	1	1	—	—
Myrtle warbler										
<i>Dendroica coronata</i>	93	10	17	1	1	424	86	148	11	18
Black-throated green warbler										
<i>Dendroica virens</i>	16	—	—	—	—	110	5	5	—	—
Blackburnian warbler										
<i>Dendroica fusca</i>	14	1	1	—	—	32	1	1	—	—
*Chestnut-sided warbler										
<i>Dendroica pensylvanica</i>	19	1	2	—	—	78	4	7	1	1
Bay-breasted warbler										
<i>Dendroica castanea</i>	12	1	1	—	—	63	1	1	—	—
Black-poll warbler										
<i>Dendroica striata</i>	6	—	—	—	—	29	1	1	—	—
*Ovenbird										
<i>Seiurus aurocapillus</i>	52	7	8	—	—	240	41	67	—	—
*Northern water thrush										
<i>Seiurus noveboracensis</i>	22	6	7	—	—	36	5	5	1	1
Mourning warbler										
<i>Oporornis philadelphia</i>	5	—	—	—	—	12	3	5	—	—
*Northern yellow-throat warbler										
<i>Geothlypis trichas</i>	36	5	6	—	—	88	24	42	—	—
*Canada warbler										
<i>Wilsonia canadensis</i>	48	6	11	—	—	91	10	13	—	—
American redstart										
<i>Setophaga ruticilla</i>	15	1	1	—	—	22	1	1	—	—
<b>Icteridae, Thraupidae</b>										
Bronzed grackle										
<i>Quiscalus versicolor</i>	26	3	5	—	—	14	1	2	1	1
Scarlet tanager										
<i>Piranga olivacea</i>	—	—	—	—	—	7	1	3	—	—
<b>Fringillidae</b>										
Rose-breasted grosbeak										
<i>Phœucticus ludovicianus</i>	14	2	3	—	—	17	3	9	2	3
Indigo bunting										
<i>Passerina cyanea</i>	1	—	—	—	—	8	3	7	—	—
Purple finch										
<i>Carpodacus purpureus</i>	40	—	—	—	—	83	2	2	1	1
White-winged crossbill										
<i>Loxia leucoptera</i>	18	2	2	2	2	20	4	6	—	—
*Vesper sparrow										
<i>Poœcetes gramineus</i>	1	—	—	—	—	15	6	13	2	4
*Slate-colored junco										
<i>Junco hyemalis</i>	42	11	15	3	3	60	23	32	17	57
*Chipping sparrow										
<i>Spizella passerina</i>	23	6	7	—	—	72	16	21	3	5
*White-crowned sparrow										
<i>Zonotrichia leucophrys</i>	7	2	2	—	—	21	1	1	2	3
*White-throated sparrow										
<i>Zonotrichia albicollis</i>	327	54	66	7	9	1065	341	660	89	137
*Swamp sparrow										
<i>Melospiza georgiana</i>	6	2	2	1	1	60	13	26	—	—
*Song sparrow										
<i>Melospiza melodia</i>	67	3	5	1	1	171	38	90	8	10
Total: (including negative birds noted in text)	1619	164	223	24	32	4829	849	1519	180	330
Per cent infested with <i>fringillina</i>			10.1					17.6		

\*Indicates birds with ground- or low-ranging habits according to Griscom (6) and Taverner (9).

Larger birds such as robins, blue jays, and saw-whet owls were kept in larger cages (2 ft X 2 ft X 2 ft) embodying the same principle as those just described. The inner surface of the cage frame was covered with 3/8-in. hardware cloth, the outer surface except the bottom with fiberglass window screen. Strips of plastic sponge attached to the bottom of the cage ensured a fly-tight seal with the tray on which the cage rested. A small sliding door on one side gave access to the cage.

Experiments to test possible preferences of hippoboscids for various species or ages of birds were carried out as follows. Two birds of different ages or species were each confined in small cages (8 in. X 8 in. X 8 in.) made of 1/2-in. mesh hardware cloth. These small cages were placed within a larger test cage (2 ft X 2 ft X 2 ft), two sides and the bottom of which were of wood; two sides were covered with nylon mesh, and the top was a sliding glass plate. Two sleeves gave access to the interior. A number of flies (from birds other than those tested) were introduced into the larger cage. The birds, at various times thereafter, were removed and examined for flies.

Search for puparia in the debris on the bottom of smaller cages necessitated the removal of the small birds from their cages. These were transferred from one cage to another as quickly as possible with a minimum of handling. The larger cages and occupants were merely moved from one tray to another. The debris was examined on a white background; the black puparia were picked up with an aspirator. Their detection was facilitated by omitting black seeds from the diet of seed-eating birds. Puparia were maintained in damp sawdust (with the addition of about 2 parts in 100 of dry boracic powder to reduce mycelial growth) in screw-capped vials. These vials were kept in incubators and water baths at various temperatures.

Scientific names of birds (Table I) are taken from the American Ornithologists' Union's Checklist of North American Birds (Fifth Edition, 1957). The birds maintained in captivity were fed the following diet. A dry mixture was formed from 1 part fish meal, 1 part brewer's yeast, 2 parts oatmeal, 2 parts soymeal, 2 parts powdered milk, 2 parts corn meal, 1/4 part bone meal, 1/4 part salt, 1/4 part powdered alfalfa. One part of this dry mixture was added to an equal portion of canned dog food; a few drops of a multiple vitamin mixture and 1-2 ounces of feed oil were also added. The ingredients, with added water, were mixed to a smooth paste. A supplemental diet of fruit was given two to three times weekly; recently, suet has been added to the cages.

The species of hippoboscids encountered in this study were identified by Professor Joseph Bequaert, at that time of the University of Houston, Houston, Texas, U. S. A. The three species, *Ornithomyia fringillina*, *Ornithoica vicina*, and *Lynchia americana* will be referred to by specific name only throughout.

### Incidence and Behavior

#### *Period of Occurrence of Hippoboscids*

Trapping and examination of birds for hippoboscids started about mid-May in 1958 and 1959. Four hundred and fifty birds were examined before the first fly was noted and thus the dates probably indicate rather accurately the first occurrence of hippoboscids in this locality. The first *fringillina* were

taken on July 12, 15, and 6, in 1957, 1958, and 1959 respectively, a month later than found by Corbet (5) in Britain but at times almost identical with those of Bequaert (3) in the New England States. The highest percentage of infested birds was taken during the last week of July and first week of August (Fig. 2). However, when migration started in mid-August, more birds were captured and thus most flies were taken in the latter half of August. Flies were captured as late as September 24 in 1959. The first *vicina* were taken on August 3 and 8 in 1958 and 1959 respectively. Most *vicina* were taken towards the end of August (Fig. 2); none were taken after September 16 in 1959, nor after October 3, in 1957, although about 100 birds were examined after these dates. As few *vicina* were taken, their occurrence is expressed as a percentage of total flies taken per week rather than as the percentage of birds infested per week as for *fringillina* (Fig. 2). *Lynchia americana* was taken on hawks and owls on October 3, in 1957, and as late as December 12 of the same year. This was the only time that hawks and owls were examined, however. Bequaert (4) believes this species breeds the year around.

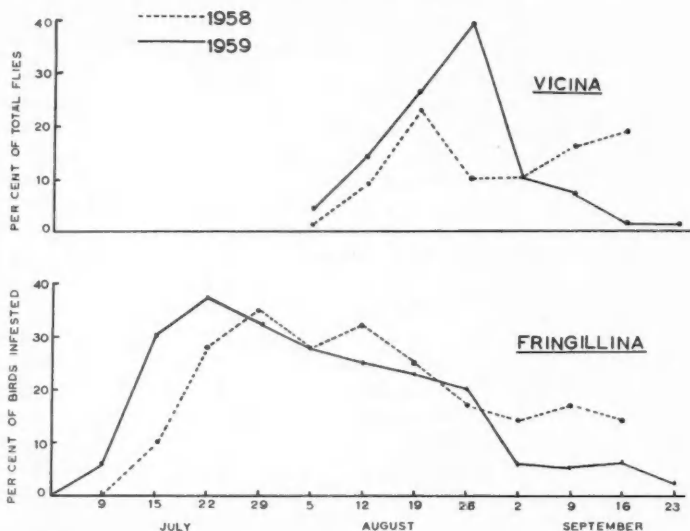


FIG. 2. Occurrence of *fringillina* and *vicina* throughout the summer. Each point on the *fringillina* curves represents an examination of 194 (47-398) birds.

#### *Incidence of Hippoboscids on Different Species of Birds*

No effort has been made in Table I to present totals and percentages for either individual species or families of birds. Use of percentages can be misleading in the treatment of data in which the number of observations vary widely. For example, 50% of the young starlings harbored *fringillina*, but only 32% of the young white-throated sparrows were infested. However, only four starlings were handled, whereas 1065 young white-throated sparrows were

examined. Similar arguments can be presented concerning use of totals for families. In addition, as will be shown later, the habits of the individual species of birds have a great effect on the rate of infestation, and to total observations of a family of birds is meaningless. Birds marked with an asterisk are considered by Griscom (6) and Taverner (9) to be birds with ground- or low-ranging habits.

*O. fringillina* was taken on 16% of 6448 birds examined between July 6 and September 24, in 1958-60 (Table I). It was recovered from 58 of the 84 species handled; Bequaert (4) lists over 100 host species. The greatest number of flies taken from individual birds was 14, 13, and 13 from an immature robin, an immature ovenbird, and an immature white-throated sparrow respectively. The following 26 species of birds, from which no hippoboscids were taken and which are not recorded in Table I, were examined during the same period.

The first figure indicates the number of adults, the second the number of immatures. TETRAONIDAE: ruffed grouse, *Bonasa umbellus* 23, 31; spruce grouse, *Canachites canadensis* 6, 3. ACCIPITRINIDAE: sharp-shinned hawk, *Accipiter striatus* 2, 3. COCCYZIDAE: black-billed cuckoo, *Coccyzus erythrophthalmus* 3, 1. APOPIDAE: chimney swift, *Chaetura pelagica* 1, 4. TROCHILIDAE: ruby-throated humming bird, *Archilocus colubris* 7, 5. ALCEDINIDAE: kingfisher, *Megaceryle alcyon* 2, —. TYRANNIDAE: crested flycatcher, *Myiarchus crinitus* —, 2; phoebe, *Sayornis phoebe* 2, —; olive-sided flycatcher, *Nuttallornis borealis* 1, 2. CETHIDAE: brown creeper, *Certhia familiaris* 3, 10. SITTIDAE: red-breasted nuthatch, *Sitta canadensis* 3, 20. MIMIDAE: catbird, *Dumetella carolinensis* 1, —; brown thrasher, *Toxostoma rufum* —, 1. VIREONIDAE: yellow-throated vireo, *Vireo flavifrons* —, 1. PARULIDAE: Parula warbler, *Parula americana* 1, 1; yellow warbler, *Dendroica petechia* —, 1; black-throated blue warbler, *Dendroica coerulescens* 13, 12; pine warbler, *Dendroica pinus* 1, 2; palm warbler, *Dendroica palmarum* 1, —; Wilson's warbler, *Wilsonia pusilla* 13, 17. ICTERIDAE: red-wing blackbird, *Agelaius phoeniceus* 1, 3. FRINGILLIDAE: goldfinch, *Spinus tristis* 6, 3; evening grosbeak, *Hesperophonia vespertina* —, 1; Savannah sparrow, *Passerculus sandwichensis* 1, 1; Lincoln's sparrow, *Melospiza lincolni* 2, 1.

Certain species of birds harbor *fringillina* more frequently than others (Table I). Among the sparrows (Fringillidae) and the thrushes (Turdidae), between 25 and 33% of the immature birds are infested. On the other hand, only about 6% of the young vireos (Vireonidae) and 5% of the young chickadees (Paridae) are infested. Such results might be expected if *fringillina* "preferred" some species of birds to others.

*O. vicina* occurred on 3.2% of the birds (28 of the 84 species (Table I), most frequently on sparrows (Fringillidae), thrushes (Turdidae), and corvids (Corvidae), a host distribution similar to that observed for *fringillina*. Fourteen flies were taken from an immature junco and 10 from a young Canada jay.

*Lynchia americana* was recovered from hawks and owls in the Toronto region; few hawks and owls were examined in Algonquin Park. Twenty-seven flies were removed from an immature great horned owl (*Bubo virginianus*).

### Host Preference

Host preference, as suggested by the data on incidence (Table I), was tested by giving the flies a choice of two birds of different species maintained in the same or separate cages within a large cage. Flies, which had no previous experience with either test bird, were released within the outer cage. The birds were examined for the presence of flies 4–12 hours later. More flies were recovered from white-throated sparrows than most other birds tested against this species (Table II). Similarly, more flies were recovered from other fringillids tested than from other groups of birds and the thrushes were "preferred" to the icterids and corvids. These results confirm the evidence for host preference suggested by the data on incidence (Table I). However, notable exceptions to the natural incidence were noted in tests with white-winged crossbills and some of the woodpeckers (Piciformes).

In several experiments, *fringillina* flew to, but did not remain on, purple finches and java sparrows. Hippoboscids were found rarely on purple finches during the survey (Table I). Tests were made, therefore, to determine if *fringillina* would starve to death rather than feed on these birds. Twenty-four "wild" and 17 reared flies died within 4 days after being placed with purple finches only. Sixty captured and 10 reared flies were placed with java sparrows; only 10 survived longer than 5 days and all were dead within 13 days. Similar tests were made using a saw-whet owl, spruce grouse, and pigeon. Thirteen "wild" and 13 reared flies survived less than 6 days when placed with a saw-whet owl; five reared flies placed with a pigeon were not recovered when the bird was examined later. Only two of 10 "wild" flies placed on a spruce grouse survived longer than 3 days. These results indicate that some species of birds are unsuitable hosts for *fringillina*. Other records (4) of *fringillina* from these birds do not indicate whether the flies were reproducing while on these hosts.

Further examination of the incidence data (Table I) shows that the ovenbird and the Northern yellow-throat warbler harbored flies more frequently than other warblers. Similarly, thrushes of the genus *Hylocichla* were more frequently infested with *fringillina* than robins. On the other hand, woodpeckers and the crossbills rarely harbored hippoboscids. Possibly the incidence is related to the habitat in which a particular species of bird occurs most frequently. For conceivably, the proximity of birds to flies at the time the latter emerge and the flight habits of the flies and the birds affect the incidence. If the puparia of *fringillina* are on the ground, flies, when they emerged, would be most numerous at ground level. Birds which frequent the ground or low levels of a forest should be in contact frequently with flies and these birds should show a higher rate of infestation. The birds (Table I) have been grouped according to their normal range above the ground as cited by Griscom (6) and Taverner (9). Of the birds living in the low to medium range, 12 to 20% were infested with *fringillina*. Only 3.5–7.5% of the birds living in the medium- to high-range habitats were infested. Among the Parulidae, 16% of the low-ranging forms were infested, compared to the 5% infestation in medium- and high-ranging forms. Among the fringillids, 26% of the low-ranging birds harbored *fringillina*, whereas only 7.5% of the high-ranging birds were infested. Clearly, the most heavily infested birds are those living on or near the ground. If, under experimental conditions, it could be demonstrated that

TABLE II  
Preference of female *fringillina* for one of a pair of birds of same age and sex but different species

Species No. 1	Species No. 2	No. flies	No. on bird 1	No. on bird 2	No. flies missing	No. trials	No. trials No. 1 better than No. 2
White-throated sparrow	Purple finch	28	15	1	12	5	5
White-throated sparrow	Java sparrow	13	10	0	3	2	2
White-throated sparrow	Downy woodpecker	41	13	12	16	7	4
White-throated sparrow	Flicker	9	4	0	5	2	2
White-throated sparrow	Robin	22	11	6	5	4	3
White-throated sparrow	Olive-backed thrush	14	7	3	4	2	1
White-throated sparrow	Cedar waxwing	15	7	4	4†	2	1
White-throated sparrow	Blue jay	11	8	3	0	2	2
White-throated sparrow	Bronzed grackle	16	11	5	0	2	2
White-throated sparrow	Song sparrow	26	10	8	8	5	4
White-throated sparrow	Junco	47	26	8	13	9	8
White-throated sparrow	White-throated sparrow	19	6	6	7	3	—
White-throated sparrow	White-winged crossbill	17	3	9	5	2	0
Robin	Bronzed grackle	22	12	8	2	2	2
Robin	Blue jay	15	8	5	2	2	2
Robin	Canada jay	22	16	0	6	3	3
Robin	Downy woodpecker	8	5	3	0	1	1
Robin	Olive-backed thrush	11	10	0	1	2	2
*Robin	White-throated sparrow	22	6	11	5	4	1
Rose-breasted grosbeak	Bronzed grackle	15	11	2	2	2	2
Rose-breasted grosbeak	Hairy woodpecker	12	7	5	0	2	1
Rose-breasted grosbeak	Blue jay	13	4	5	4	2	0
Rose-breasted grosbeak	Cedar waxwing	11	0	6	5†	1	0
White-winged crossbill	Gray-cheeked thrush	3	1	1	1	1	0
White-winged crossbill	Downy woodpecker	19	9	6	4	3	2
White-winged crossbill	Yellow-bellied sapsucker	30	12	12	6	2	1
White-winged crossbill	Cedar waxwing	12	9	1	2	1	1
*White-winged crossbill	White-throated sparrow	17	9	3	5	2	2
Downy woodpecker	Yellow-bellied sapsucker	15	2	8	5	1	0
Downy woodpecker	Flicker	13	7	2	4	2	2
*Downy woodpecker	White-throated sparrow	41	12	13	16	7	3
*Downy woodpecker	Robin	8	3	5	0	1	0
*Downy woodpecker	White-winged crossbill	19	6	9	4	3	1

TABLE II (Concluded)  
Preference of female *fringillina* for one of a pair of birds of same age and sex but different species

Species No. 1	Species No. 2	No. flies	No. on bird 1	No. on bird 2	No. flies missing	No. trials	No. trials No. 1 better than No. 2
Hairy woodpecker	Gray-cheeked thrush	7	5	2	0	1	1
*Hairy woodpecker	Rose-breasted grosbeak	12	5	7	0	2	1
Blue jay	Canada jay	5	4	1	0	1	1
*Blue jay	White-throated sparrow	11	3	8	0	2	0
*Blue jay	Robin	15	5	8	2	2	0
*Blue jay	Rose-breasted grosbeak	13	5	4	4	2	2
Java sparrow	Purple finch	23	0	0	23†	2	—
*Java sparrow	White-throated sparrow	13	0	10	3	2	0
*Flicker	White-throated sparrow	9	0	4	5	2	0
*Flicker	Downy woodpecker	13	2	7	4	2	0
*Cedar waxwing	White-throated sparrow	15	4	7	4	2	1
*Cedar waxwing	Rose-breasted grosbeak	11	0	6	5	1	1
*Cedar waxwing	White-winged crossbill	12	1	9	2	1	0
*Bronzed grackle	White-throated sparrow	16	5	11	0	2	0
*Bronzed grackle	Robin	22	8	12	2	2	0
*Bronzed grackle	Rose-breasted grosbeak	15	2	11	2	2	0
*Purple finch	White-throated sparrow	28	1	15	12	5	0
*Purple finch	Java sparrow	23	0	0	23†	2	—
*Canada jay	Robin	22	0	16	6	3	0
*Canada jay	Blue jay	5	1	4	0	1	0
*Olive-backed thrush	White-throated sparrow	14	3	7	4	2	0
*Olive-backed thrush	Robin	11	0	10	1	2	0
*Gray-cheeked thrush	White-winged crossbill	3	1	1	1	1	—
*Gray-cheeked thrush	Hairy woodpecker	7	2	5	0	1	0
*Yellow-bellied sapsucker	White-winged crossbill	30	12	12	6	2	1
*Yellow-bellied sapsucker	Downy woodpecker	15	8	2	5	1	1
*Song sparrow	White-throated sparrow	26	8	10	8	5	1
*Junco	White-throated sparrow	47	8	26	13	9	1

\* Indicate trials initially entered in the table under species No. 2.

† These flies were present on the walls of the test cage.

*fringillina* went to, and survived on, birds of medium- and high-ranging habit, it would suggest that factors other than host preference were responsible for the low natural incidence. It will be recalled that, under experimental conditions, *fringillina* went to white-winged crossbill, downy woodpecker, yellow-bellied sapsucker, and robin (all birds of medium to high range) frequently in preference to birds of lower-ranging habits (Table II). Clearly, these birds are suitable hosts for *fringillina*. The low natural incidence (Table I) recorded for them must indicate that factors other than host preference operate to reduce infestation of high- and medium- ranging birds by *fringillina*. One such factor might well be an ecological isolation on a vertical plane.

*Comparison of the Number of Flies on Adult and Immature Birds*

*O. fringillina* occurred on 17.5% of the immature birds (Table I) but on only 10% of the adult birds. Immature birds harbored an average of 1.8 flies per bird compared to an average of 1.3 flies per adult. A total of 204 birds were infested with *vicina*; 12% of these were adults, harboring an average of 1.3 flies per bird. The immature birds carried an average of 1.8 flies each. Bequaert (4) and Corbet (5) also indicate a higher incidence of these species on immature birds.

To determine whether *fringillina* preferred immature to adult birds of the same species, experiments similar to those described were carried out. In these experiments, an adult and an immature bird of the same species were used and all the flies were females. In three experiments with white-throated sparrows, 14 flies were used; eight were recovered on the immature, two on the adult, and four were missing. In three tests with white-winged crossbills, 31 flies were used; 25 flies were recovered on the immature, four on the adults, and two were lost. Similarly, in three experiments, one using juncos and two using song sparrows, 10 of the 13 flies were recovered on the immature birds, two on the adults, and one fly was lost. The adult white-throated sparrows, junco, and song sparrows often caught and ate flies. This activity biased the results in favor of the immature birds. However, assuming that all the flies lost (7 flies) were attracted to, but eaten by, the adults, the total thus recovered from the adults (15 flies) is about one-third that recovered (43 flies) from the immature birds. The *fringillina* clearly preferred the immature birds of these species. Six tests, using 34 flies, were carried out with cedar waxwings. Nine flies were recovered from the immatures, 12 flies from the adults, and 13 flies were lost. Apparently, *fringillina* preferred the adult cedar waxwings.

While maintaining flies on captive birds, it was found that only one to two flies could be kept on adults (with the exception of white-winged crossbills) for 2 or more days. If three or more flies were used, all disappeared within 2-3 days. In experiments in 1960, only four of 22 adult juncos, white-throated sparrows, and song sparrows tolerated even a single fly. White-winged crossbills were an exception to this pattern as 18-20 flies could be kept on adults for 5-10 days (probably longer, but no attempts were made). Presumably the beak of the crossbill is such that it cannot capture and destroy flies. On the other hand, as many as 12 flies could be maintained for 30-40 days on the immature birds of any of the above-mentioned species. This evidence, together

with that from the survey and experiments, indicates that *fringillina* "prefers" and has better survival on immature than on adult birds.

Data for *vicina* are not as abundant; the flies were more difficult to handle and could be overlooked. In two experiments with white-winged crossbills, more flies were recovered from the young birds, six or seven occurring on the immatures, one on the adults. In four experiments with cedar waxwings, similar to those described for *fringillina*, of the 17 flies used seven were missing, eight were recovered on immatures, two on adults. Three or four *vicina* could be maintained on adult birds for 14–21 days. Perhaps because of their small size and greater agility, they were not easily caught by the host. Our data suggest that this species is similar to *fringillina* in "preferring" immature to adult birds.

#### *Other Characteristics of Behavior*

In preliminary work, it was noted that *fringillina* went frequently to birds already carrying flies. Experiments were carried out to test this. Two or three birds, of the same age and species, were used. One carried a number of female flies prior to the experiment, a second bird (when used) fewer, and the third none. Two or three such birds were placed in one cage within the testing cage and a number of additional female flies were added. The results of these experiments (Table III) indicate that 62 of the additional flies moved to birds already carrying flies, while only 25 moved to fly-free birds. The totals are very different but there are exceptions and a final conclusion is perhaps unwarranted. Certainly, if this is characteristic behavior, it would be advantageous to the fly in nature as it would enhance the possibilities for fertilization although increasing the hazards of destruction by the host.

TABLE III

Selection by *fringillina* of birds already infested with different numbers of *fringillina*

Bird species	Total additional flies	Bird I		Bird II		Bird III No. add. flies
		No. old flies	No. add. flies	No. old flies	No. add. flies	
White-throated sparrow	5	3	1	—	—	0
	7	4	5	—	—	2
	6	3	2	—	—	0
	5	2	2	—	—	2
	6	4	1	—	—	4
	7	3	2	—	—	2
	4	2	2	—	—	0
	7	2	2	—	—	3
	4	3	3	—	—	0
Cedar waxwing	10	10	4	—	—	6
White-winged crossbill	8	8	8	—	—	0
Totals	69	44	32	—	—	19
White-winged crossbill	5	6	2	3	2	1
	13	6	6	2	2	5
	14	7	5	2	8	0
	5	8	4	2	1	0
Totals	37	27	17	9	13	6

In the experiments just described, it was found that flies, once on a suitable host, apparently did not leave it when another individual bird of the same species was introduced to the same cage. This led to the speculation that flies accustomed to living on one individual bird, if removed, might seek out and return to the same individual if given the opportunity. This hypothesis was tested as follows. Three to four "wild" females were placed with a captive bird for an arbitrary period of 3-4 days and then removed. The same bird, together with another of the same species and age, was placed in a cage into which the same flies were released. The flies were removed from the birds 8-12 hours later. In some experiments, females caught from birds other than those being tested were introduced into the same cage to act as controls. For easy recognition, one or other group of flies was marked with a spot of lacquer nail polish on the dorsum of the thorax.

In 23 tests, 51 of 80 flies returned to the bird on which they had lived previously (Table IV). Fourteen of the remaining 29 flies were not found and were presumably eaten; 15 were recovered on the new bird of the pair. In only one test were more flies recovered on the new bird than on the other and in one test the flies were distributed equally between the two. To eliminate the possibility that one bird was more attractive or a better host than the other, the following experiments were carried out. In the first experiment four females were allowed to live for 3 days on white-throated sparrow VII and then the flies were removed. This bird, together with white-throated sparrow R9, of the same age and sex, was placed in the testing cage and the flies released. All the flies were recovered from VII. The same four flies were then given no other choice but R9, for 4 days. The test was repeated as before with the addition of six "new" flies to act as controls. The first four flies were now recovered on R9, but "new" flies were distributed between the two white-throated sparrows. A similar experiment was carried out using two adult male white-winged crossbills. This experiment differed from the former in that no control flies were used. Eleven female *fringillina* were allowed to live for 3 days on bird No. 1. The flies were then removed; bird No. 1, bird No. 2, and the flies were placed in the same cage. Twelve hours later all flies were on bird No. 1. The flies were now forced onto bird No. 2 and, 3 days later, the experiment was repeated. Eight flies were now recovered on bird No. 2, three flies on bird No. 1. These results suggest, under the conditions of the tests

TABLE IV

To show the selection by *fringillina* of birds (in pairs), on one of which the flies had lived for 3 or more days prior to the test

Bird	No. tests	No. flies used		Bird No. 1*		Bird No. 2	
		(†)	Control	(†)	Control	(†)	Control
White-throated sparrow	14	38	22	22	6	5	8
Junco	1	2	3	2	1	0	2
Song sparrow	2	11	—	4	—	7	—
White-winged crossbill	4	24	—	20	—	1	—
Cedar waxwing	1	1	—	1	—	0	—
Blue jay	1	4	—	2	—	2	—

\*Indicates bird on which flies (†) had lived for 3 or more days prior to the test.

and using one species of bird, female *fringillina* return to the bird for which they had most recent experience. This implies that these hippoboscids quickly "learn" to recognize an individual bird which they can seek out, but that they also quickly "forget" the old host after living on a new one.

Four experiments to determine if males would behave in a manner similar to the females were also carried out. Reared virgin females were placed on one adult junco and reared males on another adult junco (birds in different cages) for 3-4 days. Both birds were then examined and the number of flies noted. Both birds were now placed in the same cage. The six female flies (two on each of two birds, one on each of two birds) were recovered after 8-10 days on the birds on which they had been originally placed. Of the seven male flies used (two placed on each of three birds and one on one bird), only two were recovered, both on birds harboring females. All the females produced puparia, indicating the flies had mated. Presumably males, rather than females, had moved. The possibility of the female flies moving was tested further. Two female *fringillina* were placed on each of two adult juncos, and one female on a third junco. After 3 days, each bird carrying flies was placed in a cage with another adult junco harboring no flies. Three days later the flies were recovered on the birds on which they had been originally placed. Two male *fringillina* were placed on each of two adult song sparrows and after 3 days each bird was placed in a cage with another adult song sparrow with no flies. Two of the males were recovered 3 days later on the other birds. These observations suggest that females remain on a suitable bird but males have a tendency to wander, a conclusion supported by the evidence of Corbet (5). This behavior should ensure fertilization and reduce the chances of the females being eaten by the bird.

To determine whether females would leave a bird on which they had been living and move to a more preferable bird, nine experiments were carried out using adult birds (Table V). Female *fringillina* were allowed to live for 3-4 days on song sparrows or juncos. Following this period, the birds harboring the flies were placed in the same cage with a white-throated sparrow which carried no flies (data presented previously (Table II) indicated that white-throated sparrows were the "preferred" host) for a further 3-4 days. Both birds were then checked for the presence or absence of flies. Flies were placed for 3-4 days on white-throated sparrows used in control experiments. One each of these latter were placed in the same cage with a junco, a song sparrow, and each of two white-throated sparrows which were not infested, for a further 3-4 days.

In the three tests with juncos and white-throated sparrows (Table V), the flies which had been living on the juncos were recovered on the white-throated sparrows. Flies originally on song sparrows were also recovered on white-throated sparrows, whereas flies originally on white-throated sparrows did not move to either song sparrow or junco. In the control experiments using white-throated sparrows, flies remained on the original two birds. Obviously host preference exerts a stronger influence than do the factors tending to keep a fly on an individual bird. Confinement of two different species of birds in a small cage is an unnatural arrangement however, and it does not follow that, in nature, female *fringillina* leave one suitable host for a

more suitable one. Under natural conditions the two hosts may seldom be in such close proximity.

TABLE V

Selection by *fringillina* of immature birds (in pairs) of different species, on one of which the flies had lived for 3 or more days previously

Total flies used	Birds tested	No. of flies recovered	Flies lost, presumed eaten
4	*White-throated sparrow	(4)	0
	Song sparrow	(0)	
6	*Song sparrow	(5)	0
	White-throated sparrow	(1)	
3	*Song sparrow	(1)	0
	White-throated sparrow	(2)	
3	*Junco	(0)	2
	White-throated sparrow	(1)	
3	*White-throated sparrow	(2)	1
	Junco	(0)	
3	*Junco	(0)	1
	White-throated sparrow	(2)	
1	*Junco	(0)	0
	White-throated sparrow	(1)	
5	*White-throated sparrow	(5)	0
	White-throated sparrow	(0)	
4	*White-throated sparrow	(4)	0
	White-throated sparrow	(0)	

\*Denotes the bird on which the flies lived for 3 or more days prior to trial.

The factors stimulating hippoboscids to fly to and remain on a bird are not known, but incidental observations on *fringillina* suggest motion may be important initially in guiding the fly to a host. Flies placed in an empty cage usually sat on the walls with the body parallel to the surface on which they sat. They oriented themselves to face a moving object such as a hand above the glass top of the cage. Flies, starved for a day or so, would fly to a moving object, such as a hand or piece of paper on a string, or a moving shadow, within the cage. When alerted by movement, the flies took a characteristic posture in which the thorax and head were bent backwards, as though the fly was "looking over its shoulder". When a bird was introduced into the inner cage, the flies usually oriented themselves to face the inner cage and, generally within a matter of 5 minutes, alighted on it. The flies frequently darted under the wing of the bird as it was upraised in a wing beat. Flies also landed on the back between the wings, and on the back of the head and neck, but rarely on the front of the bird. Immediately on landing, they went beneath the feathers. The whole operation took 3-4 seconds. On at least 20 occasions it was noted that the bird, especially sparrows, caught and ate the flies as they darted for the bird. On three occasions in the field the author observed a *fringillina* sitting on the end of a dead twig. The fly flew to his swinging arm, remained for 5-10 seconds and then flew away. These observations suggest that the flies are attracted by motion. Once on, or near, a bird, presumably host preference determines whether they will stay or not.

Observations were made on two groups of 12 caged *fringillina* to determine whether they would fly to moving objects in the cage. It was found that the flies always assumed the "alert" position regardless of the nature of the moving object. However, they would rarely make a flight to the object until they had been starved 8-12 hours. After this time, they flew to moving dead bird wings (song sparrow), black and white pieces of paper, and the author's hand. In every instance the flies remained on the object for 1-2 seconds only. Neither heat from a glass bottle filled with warm water, a hand in a rubber glove, nor a gentle stream of carbon dioxide elicited a noticeable response. All these observations merely confirm that motion attracts the flies initially.

*Host "Preference" of vicina and americana*

Limited information is available concerning "preferred" hosts of *vicina* and *americana*. The former was taken more frequently from birds that tend to live at medium and high ranges (Table III). A few tests with captive birds support the finding. For example, in two tests with *vicina*, nine of 15 flies were recovered on white-throated sparrows, three on downy woodpecker, and three were missing. In three other tests, rose-breasted grosbeak (four flies) was selected over grackle (one fly), white-winged crossbill (five flies) over downy woodpecker (three flies), and robin (two flies) over veery (no flies). In four other experiments (two with white-throated sparrows, two with white-winged crossbills), all nine *vicina* that had lived on one of the birds returned to the same individual. On several occasions it was found that if *vicina* were living on one bird and another bird was introduced into the same cage, the flies did not change hosts. These observations lead to the conclusion that *vicina* behaves in a manner similar to that of *fringillina*.

Some observations to establish the host preference of *americana* were made, although free-choice experiments were not carried out. Three to four *americana* were released in cages containing each of the following birds: blue jay, Canada jay, bronzed grackle, crow (*Corvus brachyrhynchos*), pigeon, white-throated sparrow, purple finch, domestic fowl (*Gallus* sp.), ruffed grouse. The flies flew to all the birds (except the white-throated sparrow and purple finch) but they remained only a few minutes before leaving. Dead flies were found at the bottom of the cages 2-3 days later. Seven flies flew readily to a spruce grouse, but within 3 days all except one fly were dead. The single fly survived for 2 weeks, during which time it produced four puparia. On the other hand, flies flew readily to and survived for 120 days or more on each of a saw-whet owl and sharp-shinned hawk. Clearly, in these tests, raptorial birds were preferred. Moreover, of the various birds examined, *americana* was obtained only from saw-whet owl, great horned owl, and marsh hawk (*Circus cyaneus*). Ninety-seven per cent of Bequaert's (4) records of this species are from raptorial birds, most of the remainder are from Galliformes. These data imply a greater degree of host specificity for *americana* than for *fringillina*.

The failure to establish *americana* readily on grouse is not surprising. The author and his colleagues have taken only a few specimens from the more than 400 grouse examined in Ontario. It was not found on 63 ruffed and spruce grouse taken between July and November in Algonquin Park, at which time

the other species of hippoboscids were present. These data support Johnson (from Bequaert (4)), who believes that *americana* is primarily a parasite of birds of prey and accidentally occurs on Galliformes. This view is contrary to that of Bequaert, who is inclined to believe that native gallinaceous birds are the original hosts, the Raptores acquiring the fly later by preying on game birds. Female *americana* were never observed flying within the cage once they became established on an owl or hawk; males were noted on six occasions. This behavior is similar to that of *fringillina*.

#### Miscellaneous Observations

As described in the methods, *fringillina* were relatively easily, often too easily, removed from birds caught in mist nets. These flies, put on captive birds, could be removed in 2-3 minutes the first two to three times the bird was handled. As the birds were handled more frequently, the flies became more difficult to dislodge. During one experiment, flies were removed from birds twice a day for 10-15 days. At the end of this period, it required 25-30 minutes to dislodge some of the flies; others refused to leave the bird. These latter would appear briefly on the surface and disappear again among the feathers. Female *vicina* behaved similarly. Whether this was "learning" or merely coincidence is not known. It adds an aggravating feature to the technique of studying these flies.

On the other hand, *americana* were difficult to remove from the outset as shown by the following observations: a great horned owl, killed when it flew into wires, was placed into a polyethylene bag the following day and examined 2 days later. Twelve *americana* were obtained from it. During the next 3 days the carcass: (i) was placed four times in a carbon dioxide-filled bag; (ii) had its feathers combed three times; and (iii) had the viscera removed. By the end of the third day, 12 more flies were recovered. The bird was now placed in a freezer unit pending its disposal. Two weeks later the carcass was again examined and three dead flies were found. Undoubtedly, the long, soft feathers of the owl obscured the flies and made it more difficult to capture them.

Phoresy of mallophagans by hippoboscids has been discussed at length by Bequaert (3). In this study only *fringillina* carried biting lice. In 1957, 41 of 180 *fringillina* carried biting lice, one female carrying eight lice; most flies carried only one or two. Phoresy was of such common occurrence that no records were kept in the ensuing years. The impression was obtained, however, that phoresy occurred most frequently in the early portion of the season (July); few flies carrying mallophagans were found during late August and early September.

#### Life History

##### Longevity

An estimate of the longevity of hippoboscids (whose history prior to capture was unknown) was obtained from flies maintained, after capture, on captive birds. Hundreds of female flies were kept for 10-20 days. Two female *fringillina* lived for 48 days and one female for each of 56, 57, 63, 65, and 81 days, after which time their carcasses were found on the floor of the cage; all flies produced puparia regularly throughout this period. Many *fringillina* were

reared from the puparia, but they emerged at a time when only adult birds were available as hosts and few flies survived when placed on them. Two reared females survived for 35 days and one for 65 days before they were presumably eaten. The results show that female *fringillina* under natural conditions and on immature birds can live for 60–90 days. Determination of the longevity of the males was more difficult as they frequently disappeared, presumably being eaten. A single male was kept for 20 days after its capture.

Two female *vicina* lived for 70 days after their capture, another pair for 79 days, and three others for 95, 111, and 126 days respectively; one reared male lived for 25 days before it disappeared. Eight reared *americana* females survived for 120–135 days while an additional 10 lived for more than 100 days. One male survived for 60 days although most of 40–50 males died in 15–20 days.

Females are apparently longer-lived than males of all three species. However, as males have a tendency to leave their hosts, this short life-span may indicate merely that they were eaten by the bird. Females of *vicina* and *americana* are apparently longer-lived than those of *fringillina*.

#### *Mating*

Mating of *fringillina* was observed on two occasions. On the first occasion, the flies were mating on the outer feathers of a myrtle warbler entangled in a Japanese mist net; they separated almost as soon as the observer approached. It was noted that the wings of the female were spread and that the male grasped the female about the thorax and abdomen on the dorsal side. The tip of the abdomen bent under the female. On the second occasion, a pair was removed from an experimental bird and placed in a vial. They remained *in coitu* for 15 minutes in the position just described, the female ran actively about the vial during this period. Four females were placed, 12 hours after emergence, on birds already carrying males. All females produced puparia within 6 days, indicating fertilization within the first 24 hours. Unmated females did not produce puparia. One newly emerged male was placed on each of two birds, each carrying a single virgin female. Puparia were produced 5 days later, indicating that mating had occurred sometime during the first 24 hours.

Female flies of all species taken from captured birds and kept isolated from males produced several viable puparia until they died. Obviously the females had been fertilized before capture and fertilization is unnecessary between the production of each puparium. Moreover, the nutrition of the developing larvae could not be derived from the abundant sperm and male secretions injected into the uterus at frequent matings, as was claimed by Berlese (in Bequaert (4)).

#### *Birth and Deposition of Puparia*

Five observations were made on the "birth" of larvae of *fringillina*. The abdomens of the females about to give "birth" were a cream color due to the developing prepupa, in contrast to the grayish-white color of females with developing larvae. The spiracles were the first part of the prepupa to appear at the tip of the distended ovipositor. No great change occurred for 10–15

minutes except that the ovipositor became very distended and the prepupa became orange-yellow. Suddenly, in about 30 seconds, the prepupa appeared on the outside and remained attached to the female at its anterior end. The prepupa remained attached to the female for 1-2 hours, during which time it became shiny and black in color and formed the puparium.

Some species of *Ornithomyia* larviposit in birds' nests (3), but the place where *fringillina* larviposits is not known. Bequaert believes that the females leave the host and larviposit. No puparia of *fringillina* or *vicina* were found in over 2500 birds' nests examined for the puparia of *Protocalliphora* (1) and Hicks (7) does not record them among the fauna of birds' nests in North America.

In the present study, over 1000 puparia of the three species of hippoboscids were collected from the cages in which birds and flies were maintained. Most of the puparia were scattered on the floor of the cage as though dropped at random from the bird. In 32 instances, puparia of *fringillina* were found floating in the bird's water dish, as were three puparia of *vicina*. In 20 instances they were found in the seed dish. On 27 occasions, puparia of *fringillina* and *vicina* fell from the bird as it was handled although the female fly was not seen at that time. Puparia were never attached to the feathers, nor have they features for such adhesion. In no instance was a puparium found in a position that suggested it had been deposited there specifically. Furthermore, experimental studies indicated that female *fringillina*, *vicina*, and *americana* do not leave a suitable host. These observations strongly suggest that the gravid females of these species do not leave their hosts to deposit their puparia in specific locations. Apparently the puparia fall from the birds at random as suggested by Corbet (5). Records cited by Bequaert suggesting specific deposition by *fringillina* can be explained just as adequately on the basis of the puparium dropping at random from the host.

#### Gestation

The period of gestation of *fringillina* was studied by maintaining known numbers of females on different birds and counting the puparia produced within a known period (Table VI). Different species of birds were used and the results varied with the different hosts. A puparium was produced in as short a time as 4.0 days by a fly on a junco and as long a period as 12 days by a fly on a java sparrow. This latter host was unsuitable as most flies did not survive on it. Generally, *fringillina* on a suitable host produced a puparium every  $5 \pm 1$  days, similar to the period found by Corbet (5).

The reproductive potential of the hippoboscids is low compared to other insects. On captive juncos, three flies each produced eight puparia in 40-42 days, one fly produced 11 puparia in 57 days, and one fly 13 puparia in 65 days. All were "wild" females whose previous history was unknown. Assuming *fringillina* will live for 75 days it could produce approximately 15 puparia, a number which would vary slightly with the host. To reproduce the species the following year, presumably two puparia or 13% of the average production would have to survive.

In a manner similar to that described above, *vicina* produced a puparium every  $5 \pm 1$  days (Table VI), the period differing slightly for flies on different

bird species. This variation was not as pronounced as that seen in *fringillina* and may indicate that more birds are suitable "breeding" hosts for *vicina*. Five "wild" *vicina* produced 9, 15, 18, 22, and 26 puparia each in 48, 81, 95, 111, and 126 days respectively.

TABLE VI

Period of gestation (to nearest half day) of *fringillina*, *vicina*, and *americana* on various captive birds

Hippoboscid	Bird species	Gestation (days)	No. of puparia
<i>fringillina</i>	White-throated sparrow	5.5 (4.0-6.0)	252
	Slate junco	4.5 (4.0-5.5)	109
	Downy woodpecker	5.0 (4.5-5.5)	10
	White-winged crossbill	5.5 (4.5-6.0)	15
	Robin	5.0 (4.5-5.5)	6
	Rose-breasted grosbeak	5.0 (4.5-5.5)	8
	Cedar waxwing	6.0 —	5
	Java sparrow	9.5 (7-12)	4
	Saw-whet owl	5.0 (4.5-5.5)	4
	Spruce grouse	6.5 (6-7)	4
<i>vicina</i>	White-throated sparrow	5.0 (4.0-5.5)	91
	Song sparrow	5.5 (4.5-6.0)	80
	Slate junco	5.0 (4.5-5.5)	31
	Saw-whet owl	4.5 (4.0-5.0)	25
	Yellow-bellied sapsucker	5.0 (4.5-5.5)	5
	Rose-breasted grosbeak	5.0 (4.5-5.5)	4
<i>americana</i>	Saw-whet owl	4.5 (4.0-5.0)	119
	Sharp-shinned hawk	4.0 —	39
	Spruce grouse	4.0 —	4

*Lynchia americana* produced a puparium in a slightly shorter period than the previous two flies, puparia being produced every  $4.5 \pm 0.5$  days (Table VI). Two reared flies produced 26 and 28 puparia in 115 and 124 days respectively. The reproductive potential of this species is slightly greater than that of the preceding two species.

Comparison of data on the gestation period of hippoboscids on various species of birds, with data on host preference (Table II), suggests that the best "breeding" host for the survival of the parasites is not necessarily the same. Although the white-throated sparrow was preferred to juncos (Table II), *fringillina* on the latter bird produced puparia in a shorter period, and thus the junco may represent the better "breeding" host. Similarly, the cedar waxwing was "preferred" to the downy woodpecker, but puparia were produced in a shorter interval by flies on the latter. The gestation of *vicina* was shortest on the saw-whet owl, which may be a good "breeding" host for this species. Data on gestation (Table VI) would be more significant if accompanied by a comparison of the survival of flies on each species of bird. However, the flies used were of varied and unknown age and such a comparison with the present data would be meaningless. Further information about the gestation period of hippoboscids on other species of birds, together with experiments as to the "preferred" host, is desirable. Such information will contribute to the concept of the best "breeding" hosts and to the population dynamics of these interesting flies.

### Metamorphosis

The rate of metamorphosis of *fringillina*, *vicina*, and *americana* at different temperatures was determined by maintaining the puparia at various temperatures until adults emerged. In all these tests, the puparia were maintained in damp sawdust at nearly saturated humidities.

Fifty-eight puparia of *fringillina* were placed in an incubator at  $82 \pm 2^\circ \text{F}$ . (Table VII, group 1) and 62 puparia were exposed at room temperature ( $75 \pm 5^\circ \text{F}$ ) until adults emerged (Table VII, group 2). Only eight adults, of which five were deformed, emerged from group 2 and none from group 1. The poor development of adults in these two groups suggested that diapause occurred.

If diapause occurred, better emergence of adults might be expected if the puparia were kept for a time at lower temperatures. Therefore, puparia were placed in a refrigerator ( $45 \pm 3^\circ \text{F}$ ) for varying periods. All puparia (Table VII, group 3) were obtained from the same females over 14 days, and were kept for 32 days at  $75 \pm 5^\circ \text{F}$  prior to placing in the refrigerator. Almost 75% of the puparia (Table VII, group 3) produced adults compared to the 13% emergence from non-refrigerated puparia (Table VII, groups 1, 2). In general, the total time for development at  $75^\circ \text{F}$  was shorter for refrigerated puparia than for the puparia at the same temperature given no refrigeration. Furthermore, the total time required for development at room temperature appeared to decrease as the time at  $45^\circ \text{F}$  increased, the success of emergence remaining about the same (Table VII, group 3). These results led to the conclusion that a period of cold temperature is essential for metamorphosis of *fringillina*, and that diapause occurs.

Duplicated tests were carried out to determine when diapause occurred in the puparia. In each series (Table VII, groups 4, 5), puparia were given a refrigeration period of 62 and 110 days for the two groups respectively. The time prior to refrigeration varied. The results (Table VII, groups 4, 5) indicate that puparia given up to 23 days at  $75 \pm 3^\circ \text{F}$  prior to refrigeration produced a high percentage of adults. As the pre-refrigeration time was increased beyond this time, the success of emergence decreased, with a suggestion that these latter adults required a longer time to metamorphose. From these results it is assumed that the onset of diapause occurs sometime within the first 23 days, when the puparia are maintained at  $75 \pm 5^\circ \text{F}$ . At the lower ground temperatures prevailing in Algonquin Park during July to September (average of  $56\text{--}65^\circ \text{F}$ ), presumably this period is longer.

As previously mentioned (Table VII, group 1), no adults emerged from 58 puparia kept continuously at  $82^\circ \text{F}$ . Fifteen puparia (Table VII, group 6) were kept 23 days at  $75 \pm 5^\circ \text{F}$ , for 100 days at  $45 \pm 3^\circ \text{F}$ , and then they were placed at  $82^\circ \text{F}$ . No adults emerged. Presumably, therefore,  $82^\circ \text{F}$  is above the upper lethal limit. Fourteen puparia maintained continuously at  $65^\circ \text{F}$  failed to produce adults (Table VII, group 7), indicating that this temperature is still too high for the successful development of diapause. Fifteen of 20 puparia given 110 days at  $45 \pm 3^\circ \text{F}$  produced adults at  $65^\circ \text{F}$  (Table VII, group 8).

The period for the development of adults of *vicina* at room temperature was 78 (36–122) days (Table VIII, group 1). Thirteen of 24 puparia held at

TABLE VII  
Time for metamorphosis of *fringillina* under various conditions of temperature

Group	Total puparia	No. emerged	No. of days			Average time at 75° F	Total time for development
			Before refig. (75 ± 5° F)	Refrigeration (45 ± 3° F)	After refig. (75 ± 5° F)		
1	58	0	—	—	—	No emergence at 82° F	
2	62	8	—	—	113	113 ( 78-137)	
3	10	4	32	14	60	102	116 (101-128)
	13	11	32	28	83	125	153 (135-172)
	35	21	32	42	77	119	161 (123-186)
	12	12	32	60	68	110	170 (141-195)
	11	7	32	84	45	87	171 (165-188)
	13	11	32	113	37	77	192 (188-205)
	13	10	32	138	32	74	212 (207-219)
	13	9	7	62	59	67	128 (103-167)
4	13	10	23	62	88	111	173 (168-219)
	22	9	33	62	58	91	153 (133-188)
	35	11	45	62	77	122	161 (123-186)
	32	21	9	110	89	98	208 (205-210)
5	36	20	14	110	80	94	204 (197-210)
	32	7	18	110	78	96	206 (204-207)
	32	3	27	110	99	126	236
	32	0	45	110	—	—	—
	15	0	15	100	—	—	No emergence at 82° F
6	15	0	15	100	—	—	No emergence at 82° F
7	14	0	At 65° F continuously; no refrigeration				
8	20	15	18	110	60 (at 65° F)	78	188 (182-202)

TABLE VIII  
Time for metamorphosis of *O. vicina* and *L. americana* under various conditions of temperature

Species	Group	Total puparia	No. emerged	No. of days			Average at 75° F	Total time for development
				Before refrig. (75 ± 5° F)	Refrigeration (45 ± 3° F)	After refrig. (75 ± 5° F)		
<i>vicina</i>	1	24	13		Continuously at 75° F		78	78 ( 36-122)
	2	1	1	13	42	93	106	138
		6	3	24	42	54	78	120 (105-133)
		1	1	42	28	38	80	108
		6	3	42	56	30	72	128 (124-133)
		8	8	56	56	22	78	134 (128-159)
		3	2	48	60	48	96	156 (139-174)
<i>americana</i>	3	40	0		Continuously at 82° F	—	—	—
	4	17	2		Continuously at 65° F	—	—	64 ( 46-82)
	5	52	46		Continuously at 75° F	—	36	36 ( 29-71)
	6	20	14	15	16	24	39	55 ( 49-63)
		1	1	10	21	31	41	62
		3	2	14	21	26	40	61
		9	4	0	30	36	36	66 ( 62-71)
		2	2	14	31	41	54	86
		2	2	21	45	15	36	81 ( 79-83)
		1	1	0	61	38	38	99
	7	30	0		Continuously at 82° F	—	—	—
8	37	15		Continuously at 63° F	—	—	67 ( 57-87)	
	27	9		Continuously at 60° F	—	—	96 ( 78-111)	

this temperature emerged. The emergence, although better than that of *fringillina* under the same conditions, was not exceptional, and there was a possibility that diapause occurred. However, refrigeration of the puparia (Table VIII, group 2) did not materially shorten or increase the duration of metamorphosis at room temperature although the success of emergence improved. In general, refrigerated puparia emerged in the same time at 75° F as did non-refrigerated puparia, the total period being extended only by that time the puparia were in the refrigerator. These data do not indicate conclusively the necessity for diapause. Forty puparia maintained at  $85 \pm 1^\circ$  F (Table VIII, group 3) failed to emerge. Only two of 17 puparia held at  $65 \pm 1^\circ$  F (Table VIII, group 4) emerged in 46 and 82 days respectively. Twenty-six puparia were kept at  $45 \pm 5^\circ$  F for 95–122 days immediately after their formation, during which period no adults emerged. The puparia were then placed at  $75 \pm 8^\circ$  F, and 19 adults emerged within 25–31 days. Presumably, metamorphosis had continued at the low temperature.

Forty-six of 52 puparia of *americana* kept continuously at room temperature emerged as adults in an average of 36 (27–71) days (Table VIII, group 5). The success of emergence suggested that diapause did not occur. However, puparia were exposed to varying periods of refrigeration. The results (Table VIII, group 6) indicate that refrigeration slows metamorphosis slightly, the total pupal period at 75° F being a little longer than that for non-refrigerated puparia. Six puparia were placed in a freezer within 2 hours of their production, two adults emerged. Thirty puparia held at 85° F (Table VIII, group 7) did not emerge. Puparia held at outdoor temperatures averaging 60 (26–90)° F and 63 (40–90)° F emerged in an average of 67 and 96 days (Table VIII, group 8).

A comparison was made between the duration of the puparial period of males and females. In this comparison, the puparial period of the two sexes was only compared between puparia in a single lot containing at least 10 puparia. In 16 lots of *fringillina* males emerged, on the average, earlier than females on 10 occasions. However, there was only about 10 days difference between them. Females among six lots emerged about 10 days before males. Males emerged before females in three of four lots of *vicina*, the difference being about 30 days. In four lots of *americana*, males emerged prior to the females by 2–4 days, probably an insignificant difference.

#### Sex Ratio

The sex ratio of reared *fringillina* during 1958 and 1959 was 1:0.65 ♀ (on 252 flies). However, the sex ratio of *fringillina* captured from birds during 1958 was 1:1.9 ♀, 1:1.5 ♀ in 1959, and 1:2.2 ♀ in 1960, giving an over-all ratio of 1:1.9 ♀ (on 1,300 flies) for all 3 years. The over-all ratio of 1:1.9 ♀ does not hold throughout the hippoboscid season. At the beginning of the season, most flies were males, the females gradually predominating (Fig. 3); in 1959, no males were taken after September 9, although females were captured. Similar results were obtained in 1960. The sex ratio of *vicina* was 1:2.2 ♀ (on 275 flies) among "wild" flies, but only 1:0.5 ♀ (on 56 flies) among reared flies. The similarity of these ratios to those of *fringillina* is striking. Too few *vicina* were taken to permit a comparison of sex ratios over the season.

Only female *americana* (38 flies) were taken from wild birds. Among the reared flies, the sex ratio was 1:1.3 ♀ (on 95 flies). The lower proportion of male *fringillina* and *vicina* in the "wild" compared to those reared reflects the shorter life span of the male. The higher proportion of males in the reared flies possibly indicates the greater hazards faced by this sex.

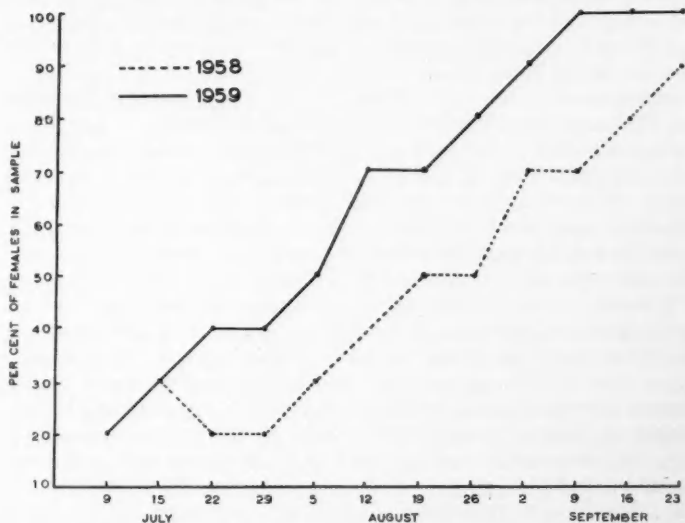


FIG. 3. To show the increasing proportion of female *fringillina* in the sample as the season progresses.

#### *Frequency of Feeding and Survival without Food*

Bequaert discusses the frequency of feeding of the Hippoboscidae and refers to the work of Tarshis on the feeding of *Stilbometopa impressa*. Tarshis (in Bequaert (3)) found that the feeding requirements of the male differed from those of the female and also reported that the female did not take a blood meal during gestation. In view of the scarcity of information on *fringillina* and the other species, the frequency of feeding of both sexes was studied.

The number of meals required by *fringillina* was determined by: (i) the period of survival without food, (ii) daily observation of flies to determine whether they contained fresh blood, and (iii) the period of gestation of flies in constant association with the host compared to that of flies on the host for limited periods.

The survival of 20 newly emerged *fringillina* in a humid atmosphere at 72-75° F but without food was determined. The flies were examined twice a day, but were maintained in the dark at other times. Males and females survived up to 5½ days but most died in 2-3 days. During the last day of life the flies were unable to crawl or fly; five flies in this condition did not survive when placed on birds. Ten *vicina*, under similar conditions and without food, survived for 3-4 days, two flies living for 5 days. Three *americana*

survived for 7 days, but seven others died in 3-4 days. These results are similar to those found by Prouty and Coatney for *Pseudolynchia canariensis* (7).

"Wild" *fringillina*, which had fed previously, were treated similarly. Five non-gravid females, filled with blood, survived for 80 (50-136) hours after removal from the host. No larvae developed *in utero*. Seven gravid females, also blood-filled, lived for 90 (72-132) hours. During this period the partially developed larvae did not increase in size nor were they re-sorbed; no puparia were produced. Seven males held under the same conditions were dead in 24-48 hours.

An estimate of the frequency of feeding of *fringillina* was determined by allowing flies access to birds for only 8-12 hours of every 24 and noting how many flies contained fresh blood when they were removed. The transparency of the cuticle facilitated this observation on 30 male *fringillina* over a 10-day period. After the first day, 26 of the flies had fed. From the second through the 10th day, every surviving male (some were eaten by the host) fed daily. Similarly, 20 females were observed over a 10-day period. An average of 18 females took a blood meal daily. These females did not produce larvae normally, however. Most females produced no larvae while the four that did required a gestation period of 8-10 days, in contrast to the 5-day gestation of flies given continual access to birds. Clearly, female *fringillina* require more than one blood meal daily to produce a puparium every 5 days. The females can survive on one blood meal a day, but under these circumstances, production of puparia is abnormally slow. Although measurements are not available, the impression was obtained that the blood meal was large when the larva was small, the size of the meal decreasing with larval growth. Possibly, therefore, at the outset of gestation, one blood meal a day is sufficient. As gestation progresses, more frequent feeding may be required to enable the intake of the same blood volume.

#### *Role as Intermediate Hosts of Blood Protozoa*

The ability of *fringillina*, *vicina*, and *americana* to act as intermediate hosts of vectors of blood protozoa of the genus *Haemoproteus* was tested. Seventeen *fringillina* were fed for 10-14 days on white-throated sparrows heavily infected with *Haemoproteus fringillae*. These flies were then allowed to live for 10 days or longer on non-infected white-throated sparrows; no transmission occurred. Nineteen *fringillina*, eight *vicina*, and 15 *americana* were allowed to live for 10-14 days on birds heavily infected with *Haemoproteus*. The flies were then examined for the ookinete, oocyst, and sporozoite stages of the parasite but none were found. Experiments presented in this paper indicate that the females of these hippoboscids studied (but not the males) rarely leave a good host unless they are disturbed. Quite probably, therefore, some females live on a single host for their lifetime. This trait, in contrast to the feeding behavior of culicids, ceratopogonids, and simuliids, would limit their usefulness and ability to act as vectors. Evidence present elsewhere (2) has shown that *Haemoproteus* is transmitted in this region prior to the time the first hippoboscids are collected. In addition, unsuitable hosts for hippoboscids have a high incidence of *Haemoproteus* (2). Obviously, therefore, these flies cannot be considered as vectors of *Haemoproteus* in Algonquin Park.

### Discussion

Experimental evidence clearly indicates that puparia of *fringillina* pass through diapause. Diapause is usually considered as an adaptation for overwintering and it is logical to assume that *fringillina* overwinters in the pupal stage. The data on incidence of infestation indicates that the adults emerge at a time of year when juvenile birds, the most suitable hosts, are most abundant in the area. The necessity for low temperature to complete diapause would explain the scarcity of this species in tropical countries. Undoubtedly some adult flies are carried to the tropics on migrating birds. However, the prevailing temperatures in such areas would not favor the establishment of a population. Significantly, the records of *fringillina* from the southern United States (3) are from migrating birds. As Bequaert states, this is a species of the temperate zones, and the data presented herein indicate a uni-voltine cycle.

Experimental evidence indicates that metamorphosis of *vicina* takes place rapidly at 75° F without diapause. Hence, two or more generations a year might be expected in Algonquin Park. However, the field data indicate that only one generation per year occurs in this region. Possibly this species, which breeds in the tropics and subtropics (3), is near the northern limit of its distribution in Algonquin Park. More data on the life history of this fly in both its northern and southern ranges are required.

*Lynchia americana* breeds throughout the year, even in mid-winter (3, 4), and data herein indicate that the puparia can withstand freezing temperatures without passing through diapause. The experimental evidence indicates that two generations a year could be expected in Algonquin Park; in warmer climates, several generations a year might be produced.

It is interesting to note that the life cycle of *americana* and *vicina* is such that under natural conditions, flies of both the first and second generation could be present on the host at the same time, thus ensuring a mixture of the gene pool. Mixing of the gene pool for *fringillina*, and to a lesser extent for the other species, can occur by a different mechanism.

Birds resident north of this area, together with the flies they are carrying, are migrating in late August and early September. En route through Algonquin Park and more southerly areas they would drop puparia, which form part of the total hippoboscids population for the next summer. Therefore, the local population of hippoboscids in any year probably includes a number of hippoboscids originating in other populations.

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**XIPHINEMA BAKERI N. SP. (NEMATODA: LONGIDORINAE)  
FROM THE FRASER RIVER VALLEY,  
BRITISH COLUMBIA, CANADA<sup>1</sup>**

T. D. WILLIAMS<sup>2</sup>

**Abstract**

*Xiphinema bakeri* n. sp. is described from soil associated with the roots of raspberry and strawberry plants. The specimens were collected near Hatzic, in the South Fraser River Valley, British Columbia. This species most closely resembles *X. index* Thorne and Allen, 1950, but is distinguished from the latter by its greater length, longer spear, more anteriorly placed vulva, and the presence in the female of only two pairs of caudal pores.

**Introduction**

*Xiphinema bakeri* n. sp. was first collected from soil around the roots of raspberry plants at Hatzic, British Columbia, Canada. The initial collection of six females, one male, and six juveniles was made by Mr. J. E. Boshier of the Experimental Farm, Canada Department of Agriculture, Saanichton, British Columbia, and was deposited as an unidentified species of the genus *Xiphinema* in the Canadian National Collection of Nematodes, Ottawa, in 1952. Subsequent examination of this material showed it to be an undescribed species. Following this, a further collection was made in 1960, in the same locality, by Mr. Boshier. Some 20 females were present in this collection and measurements, etc., showed them to be the same species.

**Materials and Methods**

Specimens from the first collection were deposited as glycerin mounts in the Canadian National Collection of Nematodes. Material from the second collection was initially preserved in modified Ditlevsen's F.A.A. (15 ethyl alcohol 95%, 6 formalin 40%, 1 glacial acetic acid, 40 distilled water), and later transferred to T.A.F. (2 triethanolamine, 7 formalin 40%, 91 distilled water). All camera lucida drawings, and other observations of specimens in the first collection, were made from glycerin mounts on Cobb slides, and from temporary T.A.F. mounts of the second collection material.

***Xiphinema bakeri* n. sp.**

*General Description*

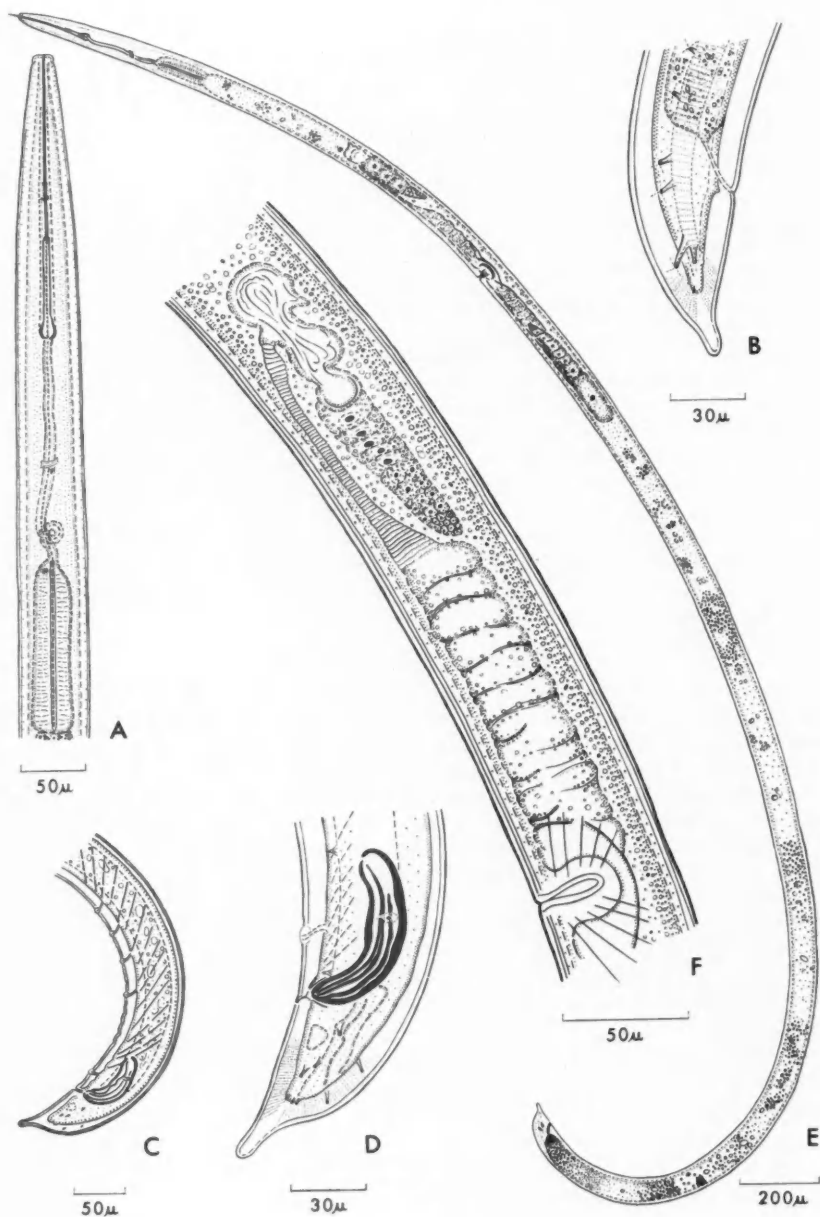
*Females* (first collection) (six specimens).—Length = 4.06 mm (3.70–4.60);  $a = 64.9$  (59.7–71.0);  $b = 8.3$  (7.9–8.7);  $c = 67.7$  (58.9–79.1);  $V = 32.7\%$  (31.5–34.3).

*Females* (second collection) (length and  $V$  values for 12 specimens, all other data for 7).—Length = 4.04 mm (3.70–4.74);  $a = 79.5$  (68.9–98.8);  $b = 8.6$  (7.1–11.4);  $c = 68.5$  (55.6–83.1);  $V = 30.4\%$  (28.3–31.8).

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There is good agreement between the de Man ratios for specimens of both collections with the exception of the *a*-values, which are higher in the second collection material. It is considered, however, that the *a*-values given for the second collection are the more reliable, since some slight flattening of the first collection specimens may have occurred. Every care was taken in the measurement of specimens from the 1960 collection to ensure that no flattening took place.

Body long, slender (Fig. 1, E), tapering slightly at the anterior end from about the level of the base of the spear extension. Width of body at this point is  $3\frac{1}{2}$  times the labial diameter. Lips six, amalgamated, lip region set off by a slight but well-marked incisure. An inner circle of 6, and an outer circle of 10 papillae present. Amphids difficult to see in lateral view but their large crescentic apertures are prominent in *en face* view.

Cuticle apparently two-layered, the outer layer showing very fine transverse striae, resolvable into series of fine dots as described by Thorne (7). The inner layer (subcuticle) exhibits longitudinal markings which are probably associated with the underlying muscle cells, since these markings are not found above the lateral chords. The subcuticle is considerably thickened around the tail, with marked radial striations which do not extend into the subdigitate tip (Fig. 1, B).

Lateral chords prominent, though there appear to be no associated lateral field features in the outer cuticle, one-quarter to one-third body diameter along most of their length, but becoming much narrower at the head end where they are only  $1/16$ th of maximum body width. The pores associated with the lateral chords form a single line from the head to the beginning of the swollen part of the esophagus, then become a double line for the remainder of the body. A complete pore count was made on three females of the second collection; in all cases the number of dorsolateral pores greatly exceeded the number of dorsoventral ones. The means of counts on the three females were dorsolateral pores 79, ventrolateral 20. The spacing of these pores was not always regular but the numbers counted on each side of the vulva agreed well with the corresponding relationships of body length, i.e., with a mean *V* value of 30%, 31% of the pores in each line lay anterior to the vulva, and 69% were posterior. Ventral pore-like structures were found along the whole length of the body, except within  $500\ \mu$  of the tail. These structures are regularly spaced, as many as 60 occurring on one specimen. The most anterior structure in this series is large and possibly represents a true pore, but though the remainder show fine processes entering the cuticle, it is not clear whether they actually open to the exterior of the worm.

Spear large (Fig. 1, A), typical of the genus, consisting of a long slender anterior portion averaging  $142\ \mu$  in length, spear extension with prominent basal flanges averaging  $77\ \mu$ . Average total length of spear and extension  $219\ \mu$  (213–226). Spear guide tubular, as described by Luc (4) and Thorne (7), giving the appearance of two guide rings  $26\ \mu$  apart. The basal portion is the most

FIG. 1. *Xiphinema bakeri* n. sp. A. Female, head and esophagus. B. Female tail. C. Male tail, general anatomy. D. Male tail, spicules. E. Female, general anatomy. F. Female reproductive system, anterior branch.

prominent and is situated  $110\ \mu$  from the front of the head when the spear is fully withdrawn.

Esophagus (Fig. 1, A) typical of the genus, averaging  $490\ \mu$  in total length, comprising a slender anterior portion which may be looped when the spear is not exerted, and a swollen basal portion, average dimensions of which are  $111\ \mu \times 32\ \mu$ . Single dorsal gland nucleus prominent at the anterior end of the basal part. The slender portion of the esophagus contains a small cuticularized structure ( $5\ \mu$  long), usually found close behind the nerve ring but variable in position and orientation. This structure has been observed in other *Xiphinema* spp., namely, *X. americanum* Cobb, 1913 (5); *X. basiri* Siddiqi, 1959 (5); *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939 (2); *X. index* Thorne and Allen, 1950 (8); *X. indicum* Siddiqi, 1959 (5); *X. insigne* Loos, 1949 (3); and *X. setariae* Luc, 1958 (4). A similar structure has also been observed by Chitwood (1) in *Xiphinemella esseri* Chitwood, 1957. The nature and function of this structure are obscure, though Chitwood and Siddiqi consider it to be associated with the possible formation of new spears in the adult.

Cardia indistinct. Intestine opaque in all specimens studied, five to six cells in circumference, well filled with food droplets (all sizes up to  $30\ \mu$  diameter). Prerectum indistinct, rectum  $28\text{--}30\ \mu$  long, surrounded by gland-like structures of uncertain relationship.

Reproductive system amphidelphic, usually more or less symmetrical with reflection occurring at the junction of oviduct and ovary (Fig. 1, F). When marked asymmetry is found in some specimens, this is due to the presence in one ovary of a large oocyte (up to  $210\ \mu$  long). When such an oocyte is present the ovary appears much longer than its opposing counterpart. If the large oocyte is absent then only a shrunken folded sac, extending beyond the junction with the oviduct, is found (Fig. 1, F). The extent of the apparent asymmetry which may result from this is seen in the range of percentage of body length occupied by the reproductive systems, which is as follows: anterior, 9.9% (8.6–11.1); posterior, 9.1% (4.5–13.5). All specimens examined showed only one prominent oocyte, suggesting that eggs are alternately released from each ovary. No sperm was observed in the reproductive tracts. Vulva transverse, with well-developed associated musculature, vagina extending almost halfway into the body. When observed from the ventral side the vagina becomes +-shaped on focussing into the body; this feature was also noted in *X. setariae* and *X. campinense* (4), and in *X. diversicaudatum* (2).

Tail dorsally convex-conoid, bearing two pairs of lateral papillae, tip subdigitate (Fig. 1, B).

*Male* (one specimen).—Length = 4.15 mm;  $a = 68.0$ ;  $b = 8.7$ ;  $c = 63.9$ ;  $T = ?$ .

In general morphology and dimensions the male closely resembles the female. Unfortunately, despite the good state of preservation of the male, no details of the testes and associated ducts were visible; the spicules and supplements, however, were fully developed.

Spicules paired,  $75\text{--}80\ \mu$  long, strongly ventrally arcuate, with a well-defined nucleate structure lying between them (Fig. 1, D). The usual pair of adanal supplements is present, plus four ventromedian ones (Fig. 1, C). Diagonal copulatory musculature is prominent. Tail similar to female in shape but bear-

ing five pairs of caudal papillae. Well-marked radial striae occur in the subcuticle near the tip, though as in the female these do not extend into the tip (Fig. 1, D).

*Juveniles* ( $n=6$ ).—Length = 2.55–3.30 mm;  $a=56.6$ –64.7;  $b=6.3$ –7.6;  $c=40.6$ –45.2.

In general form the juveniles closely resemble the adults; it would seem that changes in the de Man ratios which occur are primarily the result of increased body length in the adults. The mean juvenile tail length is, however, greater than that of the adult females ( $68\mu:60\mu$ ). Replacement spears were found in all the juveniles; these spears were fairly uniform in length ( $133$ – $138\mu$ ) and were generally about  $20\mu$  longer than the spear which they would have replaced.

#### *Differential Diagnosis*

*Xiphinema* species with the above description and dimensions, possessing a very long mouth spear and subdigitate tail, female characterized by its anterior vulva.

The most closely allied species appear to be *X. index* Thorne and Allen, 1950, and *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939. The female of *X. bakeri* differs from *X. index* in having a more anterior vulva, 28–34%:38%; by the two pairs of caudal pores (four in *X. index*); by the longer spear,  $219\mu:190\mu$ ; and also by its length, 3.70–4.60 mm compared with 3.40 mm for *X. index*. *X. bakeri* can be distinguished from *X. diversicaudatum* by its more anteriorly placed vulva, 28–34%:43–48%.

As only one male specimen of *X. bakeri* was found, the differential diagnosis must necessarily be rather tentative, particularly in view of the fact that considerable variability is now known to exist in the secondary sexual characters of males of other *Xiphinema* species. The male of *X. bakeri* might be distinguished from that of *X. index* by its much longer spear,  $224\mu:190\mu$ , the more prominent head incisures, and probably by its greater length, 4.15:3.60 mm. *X. bakeri* might be distinguished from *X. diversicaudatum* as described by Thorne (7), by its longer spear,  $224\mu:195\mu$ , but specimens of *X. diversicaudatum* described by Luc (7) and by Goodey, Peacock, and Pitcher (2) have much longer spears.

Diagnosis on the basis of male supplements and caudal pores would not seem to be too reliable in view of the variability found in these features in some *Xiphinema* species, notably *X. americanum* (6). Thus *X. bakeri* with 1+4 supplements lies within the range 1+3 to 1+5 described in *X. diversicaudatum* by Goodey *et al.* (2), and agrees exactly with the data for *X. index*. Similarly, *X. diversicaudatum* males are now known to possess three to six pairs of caudal pores; the only specimen of *X. bakeri* has five pairs. The separation of *X. bakeri* and *X. index* on the basis of caudal pore numbers, five pairs in the former, and seven in the latter, may not prove feasible since only two males of *X. index* and one of *X. bakeri* have been described, and variations in this character might well occur.

*Holotype*.—Female collected by J. E. Boshier, August 1952, collection No. 020, Canadian National Collection of Nematodes, Ottawa, type slide No. 1.

*Allotype*.—Same data as holotype. Canadian National Collection of Nematodes, Ottawa, type slide No. 2.

*Paratypes*.—Twelve females collected by J. E. Boshier, October 1960, collection No. 2124a, Canadian National Collection of Nematodes, Ottawa.

*Type habitat, host, and locality*.—Soil around the roots of raspberry plants, Hatzic, British Columbia. Paratype material from grass sod in an old strawberry growing area in the same locality.

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I also wish to thank Mr. J. E. Boshier of the Canada Department of Agriculture, Research Branch, Experimental Farm, Saanichton, British Columbia, who made the first collection of *X. bakeri*, for his kindness in making the second collection in 1960. Finally, I wish to thank Dr. A. C. Tarjan of the Citrus Experiment Station, University of Florida, for his kindness in confirming *X. bakeri* as a previously undescribed species.

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**THE FEMALE REPRODUCTIVE SYSTEM OF LONGIDORUS  
ELONGATUS (DE MAN, 1876) THORNE AND SWANGER,  
1936 (NEMATODA: LONGIDORINAE), WITH A NOTE ON  
THE GENERA LONGIDORUS (MICOLETZKY, 1922)  
THORNE AND SWANGER, 1936, AND XIPHINEMA  
COBB, 1913<sup>1</sup>**

T. D. WILLIAMS<sup>2</sup>

**Abstract**

The female reproductive system of *Longidorus elongatus* (de Man, 1876) Thorne and Swanger, 1936, is described in detail from a number of gravid females. This description is compared with the results of dissections, in saline, of the reproductive systems of young female worms and one other gravid female. The significance of a sac-like structure found at the junction of the ovary and oviduct is discussed. Certain similarities in the female reproductive systems of some species of *Longidorus* and *Xiphinema* are described, and it is suggested that further studies of these systems might assist in the clarification of certain taxonomic problems in these genera.

**Introduction**

During the study of female specimens of *Longidorus elongatus* (de Man, 1876) Thorne and Swanger, 1936, a few gravid females were found. Examination of these showed that there were some features, hitherto undescribed, the further study of which necessitated dissection of the reproductive system. Unfortunately, only one further gravid female was found, even though several thousand females were collected during the entire period of the investigation. Dissections, with the single exception of the gravid female, were performed only on young females. It is possible, though not proved, that the movement of oocytes through the reproductive tract might not be as straightforward as previously assumed. An attempt is made later in this account to relate the structure of the ovary-oviduct junction to possibly complex oocyte movements.

**Materials and Methods**

The specimens of *Longidorus elongatus* were first collected from an outdoor area of grass sod, overlying a narrow layer of sand, at the Central Experimental Farm, Ottawa, Canada. Later, two populations of the nematode (from the grass sod) were established in boxes of turf kept in a heated greenhouse. Specimens were collected as required from all three sources. The few gravid females (about 20 in all) were recovered from the outdoor population and were found only during September and October, 1959. Despite numerous prior and subsequent collections over a total period of 2 years, no other gravid females were found. A comparable situation was described by Kirjanova (3), who found very few mature females of *Longidorus elongatus* (and no males) after

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the month of May at Uzbekistan, U.S.S.R. These observations suggest that the breeding period of *Longidorus elongatus* may be confined to certain times of the year depending on local conditions. Only 15 males were collected from the Ottawa populations. In view of the large numbers of females collected it is obvious that males are relatively few in number in these populations.

The gravid females recovered were all fixed in T.A.F. (1); after relaxation by gentle heat, some were transferred to lactophenol or glycerin to facilitate observation of the internal anatomy. Slide mounts of the worms, in all three media, were prepared for microscopic examination.

With the exception of the single gravid female, only young female worms were available for dissection of the reproductive system, using the method described by Wu (8). The body wall of the living nematode was pierced near the vulva, using a fine needle, the reproductive system being effectively expelled by the high internal body pressure. These dissections were performed in a normal saline solution which minimized osmotic distortion of the delicate tissues. Temporary slide mounts, in saline, of the isolated reproductive systems were prepared, from which camera lucida drawings and observations were made.

### The Female Reproductive System of *Longidorus elongatus*

#### *The Gravid Female, Reproductive System in situ*

Both branches of the reproductive system of a gravid female are illustrated in Fig. 1, B, C. Each branch of this amphidelphic reproductive system is equally well developed in almost every female, young or gravid, though one young female was observed with a very rudimentary posterior branch consisting only of a very short, thick-walled pouch opening off the vagina. Each branch, in a gravid female, may occupy from 10–12% of the total body length.

In the gravid female, the ventral vulva ( $V=47\text{--}48\%$  for most of the Ottawa population) leads into the vagina. The vagina is strongly cuticularized and extends about half way across the body width at this point. The two opposed uteri open off the vagina via prominent arch-like cuticularized structures, each uterus averages  $250\ \mu$  in length and occupies three-quarters of the corresponding body width. The uterus terminates in a well-marked sphincter which has a powerful constricting action on eggs passing through it (Fig. 1, B, C).

Between this sphincter and the oviduct is a structure similar to the quadricolumella described by Wu (8) in *Ditylenchus destructor* Thorne, 1945, and also to a structure found in *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939, by Goodey, Peacock, and Pitcher (2). The cells of this structure in *Longidorus elongatus* appear to be multilobed and possibly secretory in nature, since their contents appear to be quite dense and finely granular when observed under a higher power than that used in preparing Fig. 1, B, C. The exact number of cells present could not be ascertained, but it appears to be greater than the 16 found by Wu in *Ditylenchus destructor*. This quadricolumellar-like structure is highly extensible to permit the passage of the very large eggs of *Longidorus elongatus*, which may attain a size of  $250 \times 40\ \mu$ . Shells were not observed around eggs prior to their passage through this region, but eggs in the uterus possessed well-developed shells.

The oviduct, which measures  $100\text{--}120\ \mu$  in length, consists of regularly

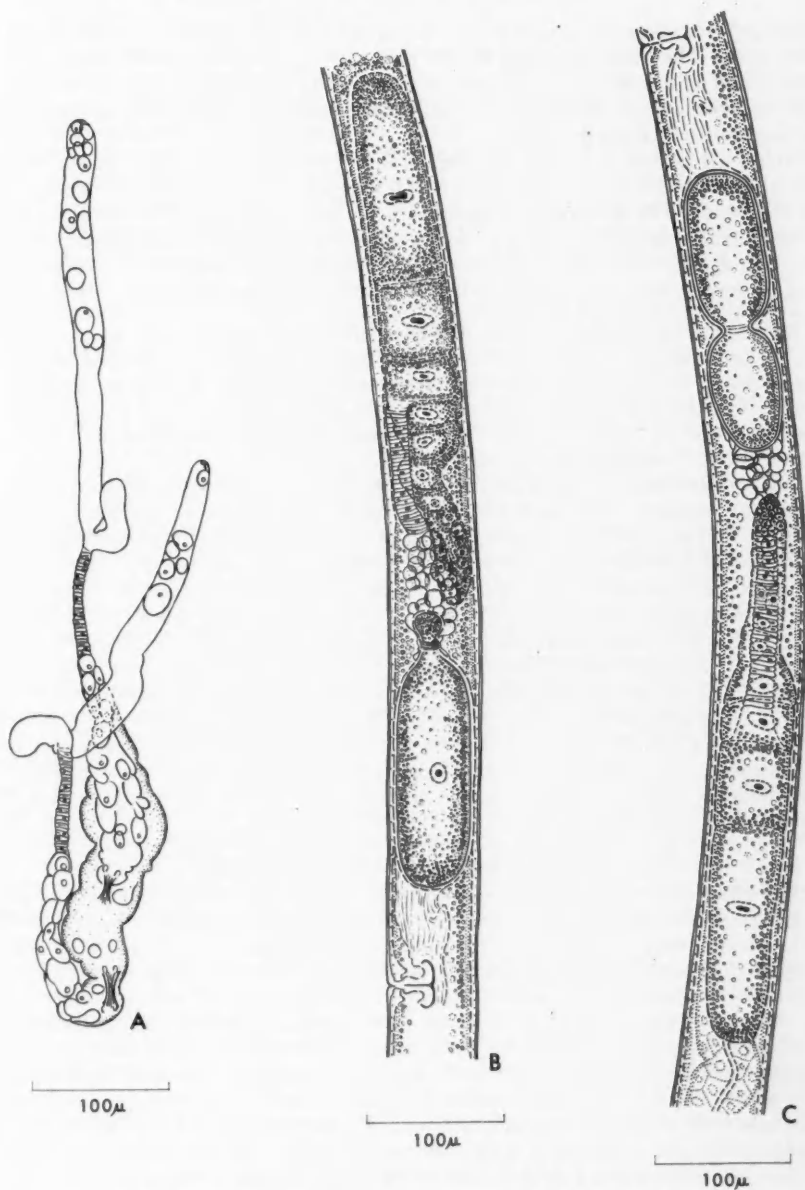


FIG. 1. *Longidorus elongatus*. A. Reproductive system dissected from young female. B, C. Anterior and posterior branches of reproductive system of gravid female *in situ*.

arranged columnar cells 15–20  $\mu$  high, and must also be capable of considerable dilatation. The apparent total length of the ovary in most gravid females is about 400  $\mu$ ; numerous oogonia are found at the germinal tip of the ovary; followed for a short distance by a double line of oocytes, then by a single row of increasingly larger oocytes.

In all whole mount specimens (Fig. 1, B, C), it was noticed that the oviduct could only be traced to a point about half way along the apparent total length of the ovary. The dissection of female worms was undertaken to establish the true relationship between ovary and oviduct. It was hoped to dissect the reproductive systems from a number of gravid females but only one more could be found despite the very large number of younger females collected.

#### *The Young Female, Reproductive System Dissected*

When dissected in normal saline solution, the reproductive system appears as shown in Fig. 1, A. It was immediately seen that a blind, sac-like structure is present at the junction of oviduct and ovary. It would seem that the presence of this structure helps to explain the apparent point of termination of the oviduct noted in undissected gravid females.

Six preparations of reproductive systems dissected from young *Longidorus elongatus* females were made in all, and each was essentially similar to that shown in Fig. 1, A. In general, the dimensions of uterus, quadricolumellar-like structure, and oviduct were similar to those found in gravid females. The ovaries, however, since they contained no maturing oocytes, were more slender in the younger females; the total distance from the germinal tip of the ovary to the end of the opposed sac-like structure varied in young females from 200 to 400  $\mu$ , the latter figure being the same as in the gravid females.

In Fig. 1, A, the lobular nature of the quadricolumellar-like regions is more evident, as are the sphincters at the junctions of these regions and the uteri. Some distortion of the uteri occurred during the preparation of the specimen illustrated, the vagina becoming detached during the dissection. The large vacuolated areas shown in the ovaries are probably osmotically induced artifacts.

#### Discussion

The presence of the sac-like structure at the junction of the ovary and oviduct prompts the question as to what function it might have. By analogy with the female reproductive systems of many other nematodes it would be tempting to designate it as a spermatheca, but no sperm was observed in any of the females examined.

Whatever the function of this structure, the movement of oocytes through this region of the reproductive tract must be considered. It appears from the examination of undissected gravid females that large oocytes occur in the area in which the sac might be located. It is unfortunate that in the only gravid female to be dissected, the sac, although 180  $\mu$  long, appeared empty and collapsed. The only large egg in this specimen had passed through the sphincter into one of the uteri, no other eggs or large oocytes were visible.

If large oocytes enter the sac, a subsequent reversal of their direction of movement is indicated, since *in situ*, oocytes move away from the tip of the

ovary and the vulva, toward the oviduct-ovary junction. Once in the sac, further progress of the larger oocyte into the oviduct would be opposite in direction to its previous movement, i.e., against the direction of oocytes maturing in the ovary. If such is the case, the flow of maturing oocytes might have to be halted until the larger oocyte in the sac enters the oviduct.

An alternative explanation might be that the flow of oocytes through the system is continuous, aided in some way by the undoubted plasticity of eggs and oocytes (shown by their deformation as they pass through the sphincter into the uterus). Very thorough observations of undissected gravid females have failed to show, however, whether oocytes overlap or flex in the sac-like structure.

Whatever the mechanism of oocyte movement, it seems that their path from the ovary to the oviduct is not as straightforward as might first appear, and that further investigation is desirable.

*Observations on the Relationship between the Genera Longidorus (Micoletzky, 1922) Thorne and Swanger, 1936, and Xiphinema Cobb, 1913*

A comprehensive study of the literature on species of both these genera, with particular reference to the female reproductive systems, enables certain observations on their interrelationships to be made.

Siddiqi (6) has commented on the overlap of characters between certain species of both genera. The occurrence of a well-developed sphincter in *Longidorus elongatus* is of interest since such a structure has been described in *Xiphinema citri* Siddiqi, 1959; the latter species possesses a guide ring more anteriorly placed than is usual in the genus *Xiphinema*. *Xiphinema brevicaudatum* Schuurmans Stekhoven, 1951 also has 'a small muscular valvular apparatus' situated between the oviduct and the uterus and this species is also considered by Siddiqi (6) to show affinities with the genus *Longidorus*, namely, in its slender body, anterior guide ring, knob-like head, and large amphids.

Further similarities can be found in the female reproductive systems of certain *Xiphinema* and *Longidorus* species. Thus, *X. rotundatum* Schuurmans Stekhoven and Teunissen, 1938 (5) is illustrated as having an oviduct-ovary junction similar to that described for *Longidorus elongatus* in this account; in addition, *X. rotundatum* has an anterior guide ring, and an anomalous spear extension lacking prominent basal flanges, more reminiscent of the genus *Longidorus*.

*Xiphinema ensiculiferum* Cobb, 1893 (Thorne, 1937), as described by Loos (4), appears to have a posterior functional ovary with an extension beyond the ovary-oviduct junction.

*Xiphinema pratense* Loos, 1949 is described by Loos (4) as having highly asymmetric ovaries occupying 6-17% of the total body length. This situation is similar to that described in *Xiphinema bakeri* Williams 1961 (7), and may likewise result from the presence or absence of large oocytes in the sac-like structure arising at the ovary-oviduct junction. This asymmetry does not appear to occur in *Longidorus elongatus* gravid females, and possibly only exists in forms where eggs are released alternately from each ovary; in almost all *Longidorus elongatus* females examined each branch of the reproductive

system contained oocytes and eggs at almost the same stages of development and position.

The comparisons listed above suggest that more detailed studies of the female reproductive systems of *Longidorus* and *Xiphinema* species would be profitable. Such studies might help in clarifying the relationships between these two genera and also assist in resolving the present uncertain status of several seemingly anomalous species in both genera.

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INFLUENCE OF TEMPERATURE AND HUMIDITY ON MORTALITY  
AND RATE OF DEVELOPMENT OF IMMATURE STAGES OF THE  
MITE *TYROPHAGUS PUTRESCENTIAE* (SCHRANK)  
(ACARINA: ACARIDAE) REARED ON MOLD CULTURES<sup>1</sup>

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Abstract

*Tyrophagus putrescentiae* (Schränk), a mite that infests stored food products, was reared individually on mold cultures at all combinations of five temperatures and four humidities. Mortality during immature stages was lowest at 72.5° F and 80% to 90% R.H. Development was usually accelerated by an increase in either of these two factors. Temperature and humidity had a direct influence on mortality and rate of development, but humidity also had an indirect effect by affecting the mold on which the mite feeds. Adults obtained at 70% R.H. were usually relatively small in size. Sex ratio of the adults was approximately one to one; the males developed faster than the females.

Introduction

Populations of *Tyrophagus putrescentiae* (Schränk) (= *castellani* (Hirst)) (6), a mite that infests stored food products, have been maintained on various cereal flakes and yeast for many years at the Entomology Research Institute for Biological Control, Belleville, Ontario. Because these mites are used in predator-prey interaction studies (1), it was necessary to obtain detailed information on their life history, behavior, and ecological requirements. Early in the investigations it was observed that the mites fed on molds growing on the food medium rather than on the food material itself, and a technique was developed for rearing the mites individually on molds and without contact with the food medium (3).

The results of earlier experiments at 77° F (4, 5) showed that the humidity had a direct influence on the rate of increase of the mite by affecting its development, survival, and fecundity and an indirect influence by affecting mold growth. No development occurred at 60% R.H., and the maximum rate of population increase was obtained with 90% R.H. The effects of various combinations of temperature and humidity on the mortality and rate of development of the immature stages of the mite are reported and analyzed here.

Materials and Methods

The mites used in these studies were reared individually and were from stock cultures bred for several generations on a mold, *Aspergillus* sp. The rearing cells were stacked in trays and placed inside desiccator jars. Fairly constant humidities of 70%, 80%, and 90% R.H. were maintained in the different jars with approximately 150 cc of appropriate solutions of sulphuric acid (7). Periodic checks with a hair-hygrometer showed that the humidities were within 2% of the percentage desired. These solutions were replaced with fresh

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ones every 5 or 6 weeks. Humidities close to 100% were obtained by using distilled water rather than an acid solution. The desiccators were placed in cabinets maintained at constant temperatures of 68.0°, 72.5°, 81.5°, and 86.0° F, with variations of  $\pm 1^\circ$  F. Each morning the cells were removed to a room at a temperature of approximately 74° F for the time necessary to make the observations with a stereomicroscope.

Twenty replicates of 10 eggs incubated individually were used in each test, and the development of the survivors was recorded daily. All eggs were not more than 24 hours old at the beginning of each test and, whenever possible, those within a replicate were obtained from a single female. Mites were disturbed as little as possible throughout their development. However, if fungal growth was not satisfactory, the individual was transferred to a new cage. If growth was too dense for easy observation, the cage was opened and part of the mycelium removed. The amount of time and quantity of material required for these experiments limited to five or six the number of replicates that could be run concurrently.

The percentages of mortality observed in the development of *T. putrescentiae* have been calculated to the closest half-unit and the average duration of the various stages to the first decimal. Only the data on the individuals that reached the adult stage were used to determine these durations; those that died or were lost during the experiment were not included in the analysis. Data for rearings at 77° F were obtained from previous studies (4), and some transformation of the figures for larval and nymphal mortalities was needed before they could be incorporated in the present paper. Except when otherwise indicated in the text, the "t" test was used to determine significance at the 5% level (2).

## Results and Discussion

### Mortality

Mortality during incubation of the eggs was lowest at 72.5° F for all the humidities tested, except at 100% R.H. where it was lowest at 68° F, though not significantly different from that at 72.5° F (Table I). There was also no significant difference in the egg mortality at 77° and 81.5° F, and it did not differ significantly even at 86° F when the humidity was 100% R.H. The effects of humidity on mortality varied with the temperature. The lowest mortality at 68° and 72.5° F was at 80% R.H., whereas at 86° F this occurred at 100% R.H. Except at the latter temperature, however, maximum mortality always occurred at the highest humidity. The effects of different relative humidities were not significantly different at all temperatures. The percentage mortality at 81.5° F was approximately equal at all four humidity levels. At 68°, 77°, and 86° F it was significantly different only at 90%, 100%, and 70% R.H. respectively. At 72.5° F the percentage of eggs failing to hatch was about the same at 70% and 80% R.H. but increased significantly at humidities of 90% and 100% R.H. Dissection of samples of unhatched eggs at 68°, 72.5°, and 86° F and all four humidities showed that less than 50% of the eggs developed, but no correlation between fertility and temperature or humidity could be shown.

Mortality in the larval stage was similar to that in the egg stage in that it

TABLE I  
Percentage mortality of immature stages of *T. putrescentiae* at various combinations of temperature and humidity

Temp. (°F)	R. H. (%)	Egg*	Larva	Protonymph	Deutonymph	Egg to adult
68.0	70	20.5	14.5	5.5	5.0	40.0
	80	18.5	5.0	2.0	1.5	26.0
	90	31.0	1.5	0.5	0.0	33.0
	100	26.0	6.0	3.0	0.0	32.5
72.5	70	12.5	10.0	8.0	0.5	29.0
	80	9.5	2.0	4.5	0.0	15.5
	90	23.0	0.5	2.5	0.0	26.0
	100	28.0	22.5	6.0	0.0	47.5
77.0	70	37.5	19.0	16.0	0.0	57.5
	80	36.5	6.5	15.0	0.0	49.5
	90	33.0	10.5	15.0	0.0	49.0
	100	44.5	23.5	24.5	1.5	68.5
81.5	70	39.5	31.0	21.5	0.0	68.5
	80	40.0	23.5	6.0	0.0	58.0
	90	38.0	21.0	13.0	0.0	58.0
	100	45.5	31.5	6.0	0.0	66.5
86.0	70	70.0	87.5	100.0	—	100.0
	80	56.0	40.0	16.5	0.0	79.0
	90	51.0	38.5	20.5	0.0	77.5
	100	49.5	24.5	6.0	0.0	67.5

\*Two hundred eggs incubated in each test.

was also lowest at 72.5° F over the whole humidity range, except at 100% R.H. where it was significantly lower at 68° F (Table I). At this high humidity, however, larval mortality was approximately the same in the temperature range 72.5° to 86° F. In relation to humidity, lowest mortality of the larvae was at 80–90% R.H., except at 86° F where it was at 100% R.H. Highest mortality, on the other hand, occurred at 70% R.H. when temperature was 68° or 86° F, and at 100% R.H. when it was 72.5°, 77°, or 81.5°, though at the latter temperature no significant difference was found between the results obtained at the various humidities.

Fungal growth was usually very dense at 100% R.H. The extra handling then required to remove part of the mycelium could have been a factor contributing to the loss of some individuals. This might account partly for the relatively high mortality observed for the eggs and larvae at this humidity.

Percentage mortality of the protonymphs at each relative humidity generally increased with the temperature, though within the range 70% to 90% R.H. the increases were not significant between 68° and 72.5° F and between 77° and 81.5° F (Table I). At 100% R.H., however, the mortality was significantly higher at 77° F. Lowest mortality occurred at 90% R.H. when temperature was 68°, 72.5°, or 77° F, and at 100% R.H. when it was 81.5° or 86° F. With the exception of 77° F and 100% R.H., mortality was highest at 70% R.H., and at that low humidity no mites survived beyond the protonymphal stage at 86° F. For any given temperature below 86° F, however, there was

no significant difference between the percentage mortality at 80%, 90%, and 100% R.H.

Very few individuals died in the deutonymphal stage. The mortality which did occur was usually at combinations of low temperatures and humidities (Table I).

The total percentage mortality of the immature stages was generally lowest at 72.5° F and increased with temperature, except at 100% R.H. where it was lowest at 68° F, reached maximum at 77° F, and remained relatively constant at the higher temperatures (Table I). An increase in temperature from 77° to 81.5° F, however, did not significantly increase the percentage mortality. Though immature mortality was usually lowest and approximately the same at 80% and 90% R.H., it tended to decrease with increasing humidity when temperature was also increased. It was highest at 70% R.H. under both extremes of temperature. In these experiments, highest survival of the mite was obtained at 72.5° F and 80% to 90% R.H. No mites developed to the adult stage at 86° F when the humidity was maintained at approximately 70% R.H.

The proportion of adult males and females surviving varied with each combination of temperature and humidity. A chi-square test, however, showed that these factors did not significantly influence the percentage survival of the sexes ( $P > 0.05$ ).

#### *Rate of Development*

Development of the eggs was accelerated by an increase in temperature (Table II). A rise of approximately five degrees was sufficient to shorten the length of the incubation period significantly. The eggs also hatched more rapidly at higher humidities, but significant differences between the time of hatching at each humidity were obtained only at 72.5° F. At all other temperatures, development was considerably retarded at 70% R.H. but little affected by a change in humidity within the range 80% to 100% R.H.

The time spent in the larval stage was usually shortened by an increase in temperature (Table II). Development was significantly lowest at 68° F in each test. At 70% R.H. the larvae developed in about the same length of time at both 72.5° and 77° F. There was also little difference in development between 81.5° and 86° F and between 77° and 86° F at 80% and 90% R.H. respectively. At 100% R.H. development was almost unaffected by an increase in temperature from 72.5° to 81.5° F. A higher humidity generally increased the rate of development of the larvae though differences between all humidity levels were significant only at 68° and 72.5° F. At 77° and 81.5° F and within the ranges 90% to 100% R.H. and 80% to 100% R.H. respectively, the rate of larval development was similar. At 100% R.H. and 86° F, however, a significant reduction was obtained in the time spent in this stage.

The protonymphs also completed their development more rapidly at the higher temperatures (Table II). The time required was significantly longer at both 68° and 72.5° F and with humidities of 90% R.H. or lower. At 100% R.H. there was a significant increase in time of development at 68° F only. As in the larval stage, a higher humidity increased the rate of development. Again the differences between all humidity levels were significant only at 68° and 72.5° F. At higher temperatures, however, duration of the protonymphal stage was

TABLE II  
Duration of various stages in days in the development of *T. putrescentiae* at different combinations of temperature and humidity

Temp. (°F)	R.H. (%)	No. mites*	Egg		Larva†		Protonymph†		Deutonymph†		Total (egg-adult)						
			Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.					
68.0	70	112	7-14	8.9	1.0	3-19	7.3	4.0	3-23	8.1	4.3	2-23	11.5	5.9	21-62	35.8	8.1
	80	143	7-12	8.4	1.1	2-12	5.9	1.5	3-13	5.6	2.2	3-21	7.4	3.4	18-46	27.3	5.6
	90	133	6-12	8.2	1.2	2-9	4.0	0.9	2-7	2.7	1.0	1-5	4.0	0.7	13-28	17.7	1.7
	100	134	6-12	8.0	1.3	2-7	3.6	0.8	2-6	2.7	0.7	1-5	3.4	0.7	14-24	17.7	1.7
72.5	70	135	6-11	7.1	0.9	3-12	4.4	1.6	2-18	5.0	2.7	3-31	7.9	4.5	15-56	24.4	7.1
	80	166	5-10	6.8	0.9	2-8	3.7	0.9	2-7	3.1	1.0	2-11	3.8	1.1	14-31	17.4	2.6
	90	148	5-8	6.1	0.5	2-6	3.1	0.7	2-7	2.5	0.8	1-6	2.6	0.8	12-20	14.3	1.3
	100	98	4-9	5.9	1.0	1-4	2.8	0.7	1-4	2.0	0.6	1-4	2.3	0.7	11-17	13.0	1.6
77.0	70	85	5-8	6.1	0.8	3-7	4.5	0.9	2-7	3.8	1.2	2-14	4.5	2.0	14-31	18.9	3.2
	80	101	4-7	5.5	0.6	2-6	3.1	0.7	1-6	2.8	1.0	1-9	2.8	1.0	11-22	13.9	2.1
	90	102	4-7	5.5	0.6	2-4	2.5	0.6	1-5	2.0	0.6	1-5	2.2	0.5	10-20	12.2	2.1
	100	63	4-7	5.3	0.6	2-6	2.7	0.8	1-5	2.1	0.8	1-6	2.5	0.9	10-21	12.6	2.0
81.5	70	59	4-7	5.4	0.6	1-7	3.2	1.3	2-7	3.6	1.3	2-7	4.2	1.4	12-23	16.4	2.5
	80	80	4-7	5.2	0.6	2-5	2.7	0.8	1-4	2.3	0.6	1-6	3.0	1.0	11-20	13.2	1.8
	90	82	4-7	5.1	0.7	1-8	2.6	1.0	1-3	1.8	0.5	1-6	2.2	0.7	9-17	11.7	1.5
	100	64	4-6	5.0	0.7	1-5	2.6	0.7	1-4	2.0	0.8	1-4	2.1	0.5	10-17	11.7	1.4
86.0	70	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	80	40	4-6	4.9	0.6	1-4	2.6	0.6	1-5	2.6	0.9	1-5	3.7	1.6	11-20	13.8	2.1
	90	43	4-6	4.6	0.6	1-4	2.6	0.7	1-4	1.9	0.7	1-7	2.4	0.9	9-14	11.5	1.5
	100	61	4-6	4.8	0.6	1-4	2.2	0.6	1-5	1.8	0.7	1-5	2.0	0.7	9-14	10.8	1.2

\*Number of individuals that reached the adult stage.  
†Including the quiescent period before molting.

approximately the same at 90% and 100% R.H.

Development of the deutonymphs was longest at 68° F and was shortened with increase in temperature up to 77° F (Table II). Above that the duration of the stage was practically constant, except at 100% R.H. where an increase from 77° to 86° F did significantly increase the rate of development. The time spent in the deutonymphal stage was also shortened with increase in humidity, but there was no significant difference between 90% and 100% R.H. at 77° and 81.5° F.

Before each molt the mite passed through a quiescent period that lasted from a few hours to more than three days. This period tended to be longer at combinations of low temperatures and humidities. Its duration also seemed to vary inversely with the length of the feeding period preceding the molt. Because the material was examined only once daily, some variation may have resulted from a failure to observe the exact time of initiation or termination of the quiescent period.

The influence of temperature and humidity on the immature stages was reflected in the time *T. putrescentiae* required to reach the adult stage. An increase in either of these factors usually increased the rate of development of the mite, though the differences were not always significant. For instance, raising the temperature from 77° to 86° F at 80% R.H., from 81.5° to 86° F at 90% R.H., or from 72.5° to 77° F at 100% R.H. was not sufficient to shorten the development period significantly. At 70% R.H., however, a rise of 10 degrees was sufficient to cut the time required for development in half. Raising the humidity from 90% to 100% R.H. at either 77° or 81.5° F also gave no significant differences.

The adults obtained from rearings at 70% R.H. were almost invariably smaller than those produced at a humidity of 80% R.H. or higher. No marked difference in size was found in the individuals reared at different temperatures. This could probably have resulted from the desiccating effect of low humidity on both the mite and mold. Fungal growth was rather slow and relatively more spores were produced at 70% R.H. As the larvae and nymphs apparently preferred to feed on the mycelium, a less palatable food produced under drier conditions might have affected the development of the mites without influencing their survival too much.

Males usually developed faster than the females (Table III). The differences observed were greatest at combinations of low temperatures and humidities, and they were significant at the 1% level at 68° and 72.5° F. Though the proportion of males and females varied with the conditions of the experiment, there was no influence of temperature or humidity on the sex ratio, which was approximately 52% females for the total number of individuals reared. A chi-square test proved that this is not significantly different from an expected one-to-one ratio ( $P > 0.05$ ).

The results obtained in these experiments indicate that, while temperature influenced the percentage mortality and the rate of development of the mite directly, it had little effect on the mold, except perhaps at 86° F; then production of spores was accelerated to the detriment of mycelium. Humidity, on the other hand, though affecting mortality and rate of development directly, also



had an indirect effect by influencing the amount and probably the quality of the food available to the mites.

Considering only the influence of temperature and relative humidity on survival and the time required for one generation, it is difficult to determine exactly the limits of the physical conditions most favorable for population increase. The latter depends largely on a high rate of reproduction. This in turn is influenced by the longevity and fecundity of the adults. Results of further experiments designed to investigate the latter aspect will be reported and discussed in a forthcoming paper.

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## THE ACTION OF FUMIGANTS ON INSECTS

### I. THE UPTAKE OF HYDROGEN CYANIDE BY *SITOPHILUS GRANARIUS* (L.) DURING FUMIGATION<sup>1</sup>

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#### Abstract

The rate of uptake of gaseous hydrogen cyanide by *S. granarius* has been measured and shown to increase linearly during exposure, even after a lethal dose has been sorbed. The sorbed fumigant can be divided into a recoverable and an irrecoverable fraction, and some of the recoverable fumigant disappears from surviving insects during the recovery period, but over 80% of it remains in their bodies for at least 4 days. Attempts to relate the disappearance of fumigant with detoxication mechanisms such as are present in mammals were unsuccessful.

#### Introduction

One important factor in the toxic action of any insecticide involves the rate and degree of uptake of the toxicant. This factor is particularly significant with gaseous insecticides because most fumigation procedures allow the insects to be exposed for only a limited length of time; the lethal effects are then governed by the total uptake during this time.

There is not much known about the way in which fumigants are taken up by insects. Some studies, made on the sorption of hydrogen cyanide, have shown that the least tolerant insects took up more fumigant per unit time than those that were most resistant (1, 2, 4, 8). Tolerance was therefore generally interpreted as the ability of the insects to exclude the fumigant from the body. It was also shown that after treatment a certain proportion of the sorbed fumigant could be recovered from the tissues, and in scale insects more could be recovered from susceptible than from resistant individuals (4). Quantitative determinations on the amounts of sorbed and recoverable hydrogen cyanide have been made on several species of insects (8) and under varying conditions of pressure and concentration (1). All of this work has been concerned with the relative amounts of fumigant taken up by the insects under different conditions to produce lethal effects. This present study was designed to find out how the fumigant was taken up by the insects and to investigate some of the effects which brought about the death of the insects. Such factors as the rate of uptake of the fumigant, the response of the insect, and the ability of the insect to detoxify the fumigant, which are important for the interpretation of toxicological effects, were studied.

The rate of uptake of hydrogen cyanide by *Sitophilus granarius* (L.), at one initial concentration, was determined, along with the amount of reversibly held fumigant subsequently found in the insects after treatment. The disappearance of reversibly held hydrogen cyanide from the insects after they

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had been treated was also studied and it was found that this disappearance coincided with recovery of survivors from the effects of treatment. Attempts were made to relate this disappearance of hydrogen cyanide with detoxication pathways known to remove toxic amounts of cyanide from the bodies of mammals.

### The Uptake of HCN by *Sitophilus granarius* during Fumigation

#### 1. Apparatus and Procedure for Fumigating the Insects

The apparatus used for exposing the insects to the fumigant (Fig. 1) consisted essentially of two contiguous chambers, a gas chamber (B) into which a given amount of gas could be introduced and an exposure chamber (E) which contained the test insects. A three-way tap (A) provided openings for evacuation of the gas chamber and introduction of the fumigant to it. A glass socket (D) with its axis perpendicular to the gas chamber held the exposure chamber which was in the form of a hollow tap plug. This arrangement allowed the exposure chambers to rotate and connect with the gas chamber B through the 1/2-in. hole F. By turning the chamber E further, it could be connected to an aspiration system at G and aerated separately. A small stirrer (C), made of a disk of aluminum set into a 1/2-in. length of stainless steel wire, was oscillated along the length of the gas chamber with an external magnet to assist in transferring the fumigant from the gas chamber to the exposure chamber. This apparatus was connected, by means of capillary tubing, to a 50-l. reservoir containing a previously prepared concentration of fumigant. A sampling tube was similarly connected to the flask so that they could both be evacuated and filled with the same concentration of fumigant simultaneously. Analysis of the contents of the sampling tube was thus used to determine the initial concentration present in the apparatus.

Fumigant was applied by evacuating the gas chamber and the sampling

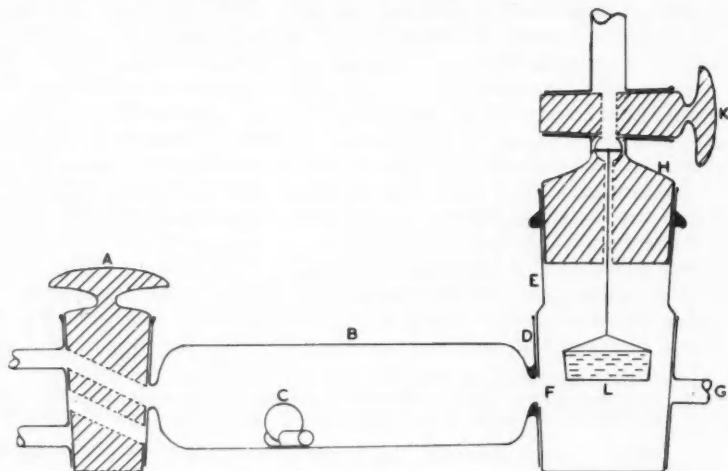


FIG. 1. The fumigation chamber.

tube to 0.5 mm of mercury, then turning the taps to connect with the capillary tubing leading to the reservoir. Three minutes were allowed for pressure to reach equilibrium between the two chambers and the reservoir, then the taps were closed. The exposure chamber of the fumigation apparatus, which contained the insects, was then turned to connect with the gas chamber and stirring was effected to mix the atmospheres of the two chambers. Analysis of the fumigant showed that mixing was complete within 10 minutes. The fumigant in the gas sampling tube was analyzed and, from this analysis, the initial concentration to which the insects were exposed was determined.

## *2. Collection and Analysis of the Fumigant*

The residual fumigant remaining in the atmosphere at the end of the exposure was collected by drawing a stream of carbon-dioxide-free air through the fumigation apparatus and thence through two bubblers in series. Each bubbler contained 5 ml of 0.04 *N* sodium hydroxide solution. Aeration at a flow rate of 50 ml per minute was found to remove all free hydrogen cyanide from the apparatus and retain it in the first bubbler.

Hydrogen cyanide concentrations were determined by the starch-iodine titration method. The solution containing sodium cyanide was removed from the bubbler and made up to standard volume of 10 ml. One milliliter of this solution was transferred by means of a washout pipette to a 2-cm rectangular colorimeter cell, and 0.5 ml of a 7% sodium bicarbonate solution and 0.2 ml of a 1% starch solution were added. The iodine solution was 0.02 *N* and was delivered from an Agla micrometer syringe mounted horizontally on a vertical rack mechanism. The solution was stirred during the titration by the electromagnetic rotation of a small iron bar enveloped in glass. The end point of the titration was judged by comparison with a colorless solution and one containing Evans blue dye, which matched the starch-iodine color. These two solutions were placed on the stirrer platform on each side of the test solution. The best form of illumination was found to be light reflected from a filter paper surface.

## *3. Recovery of Hydrogen Cyanide from Fumigated Insects*

When insects are treated with hydrogen cyanide a certain proportion of the sorbed fumigant combines irreversibly with body components and is not recoverable whilst the remainder is held loosely and can be recovered by hot aeration.

The method used for recovering hydrogen cyanide from insects was similar to that used by Lubatti (5) in recovering hydrogen cyanide from fumigated wheat. After treatment the insects were aerated and recoverable cyanide was collected by drawing air through a soda lime tower into a test tube containing the insects suspended in water, then through a reflux condenser to a bubbler containing 5 ml of 0.04 *N* sodium hydroxide. The test tube containing the insects was placed in a boiling water bath and aeration was continued for 45 minutes at a flow rate of 50 ml per minute.

This method of removing hydrogen cyanide from the insects was found to be as good as or better than previously reported methods (1). Crushing the insects did not increase the yield of hydrogen cyanide and lowering the pH of the brei resulted in a decreased yield.

### The Uptake of Hydrogen Cyanide by *Sitophilus granarius*

*S. granarius* adults were exposed to hydrogen cyanide at an initial concentration of 8 mg/l. for periods of 0.25, 0.5, 1, 2, 4, 6, 8, 14, and 20 hours and both the total fumigant sorbed and the amount recoverable from the insects were determined. As Fig. 2 shows, the rate of uptake decreased with exposure but it should be noted that the fumigant concentration about the insects also fell simultaneously. This undoubtedly had a considerable influence on the rate of uptake, and indeed, produced a false impression of approaching equilibrium and saturation of fumigant in the tissues. Lubatti (6, 7) showed that this illusion of equilibrium could be largely compensated for by converting the sorption values obtained to the corresponding values that would have been obtained in a fumigation with a steady concentration. When this correction was applied to the sorption of hydrogen cyanide by wheat the shape of the sorption curve changed from one indicating apparent equilibrium to one that was exponential in nature.

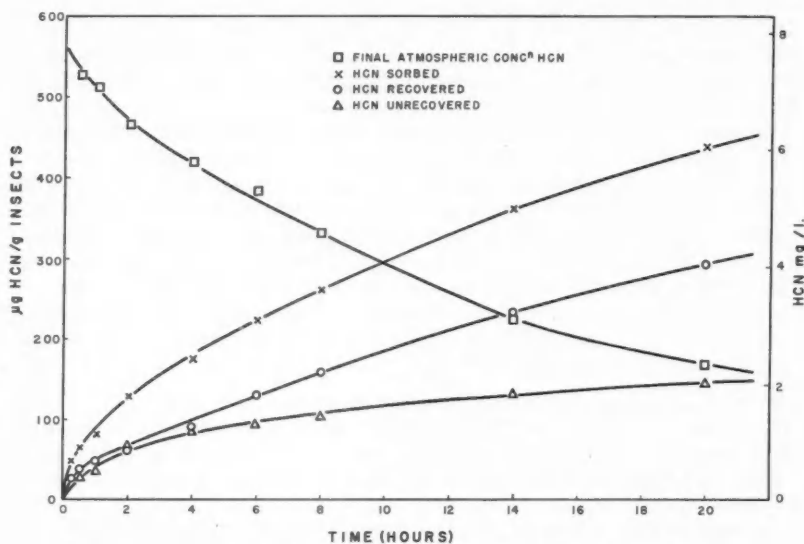


FIG. 2. The amount of HCN in the insects at various intervals after exposure to a falling concentration of fumigant.

When the results obtained from determinations on insects were corrected in this way, the sorption curve and the curve representing recoverable hydrogen cyanide became linear (Fig. 3). The linearity of the sorption curve suggested that hydrogen cyanide was being fixed in the body components of the insects and that the rate of sorption was dependent on the rate of fixation rather than on the rate of diffusion. The rate of uptake was further investigated by exposing insects to a constant concentration and measuring the amount of recoverable fumigant held in the tissues.

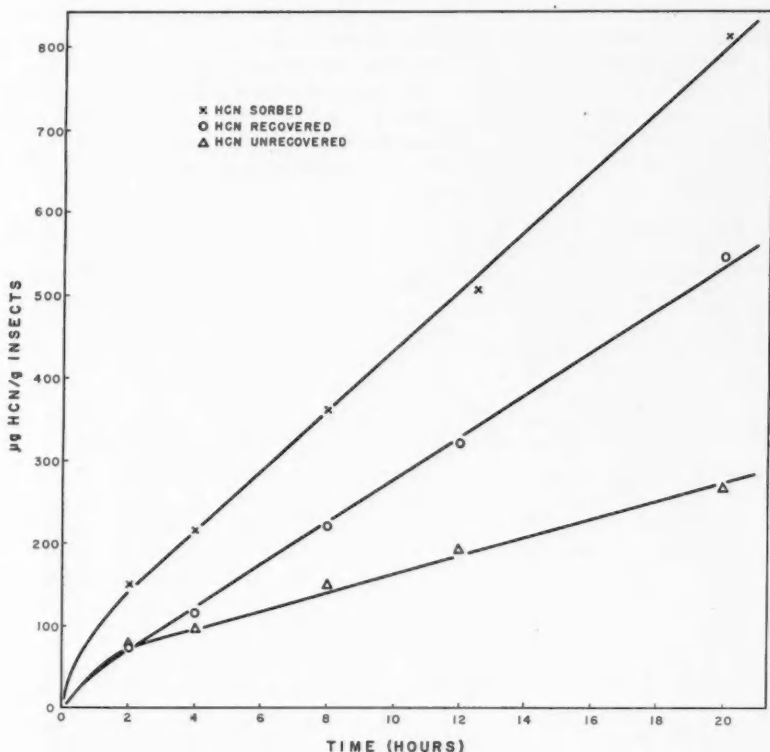


FIG. 3. The calculated uptake of HCN when the insects were exposed to a constant concentration of 8 mg/l.

*S. granarius* adults were exposed to a constant concentration of 7 mg/l. of fumigant for varying periods of time up to 96 hours and analyzed to determine the amount of recoverable hydrogen cyanide present. The results, which are shown in Fig. 4, indicated that the concentration of recoverable hydrogen cyanide increased linearly just as was found from those results calculated for a steady concentration (Fig. 3).

#### The Disappearance of Reversibly Held Hydrogen Cyanide from Insects after Fumigation

The levels of loosely held hydrogen cyanide present within the insects at various periods after treatment were investigated to determine whether this fraction remained or was removed as the insects began to recover from paralysis. As Table I shows, there was a steady decrease in the quantity of recoverable hydrogen cyanide during the first 12 hours after treatment, and at 20 hours there was only 76% of the original amount present. It is during this 20-hour period that survivors of such a treatment recover from paralysis. Because the

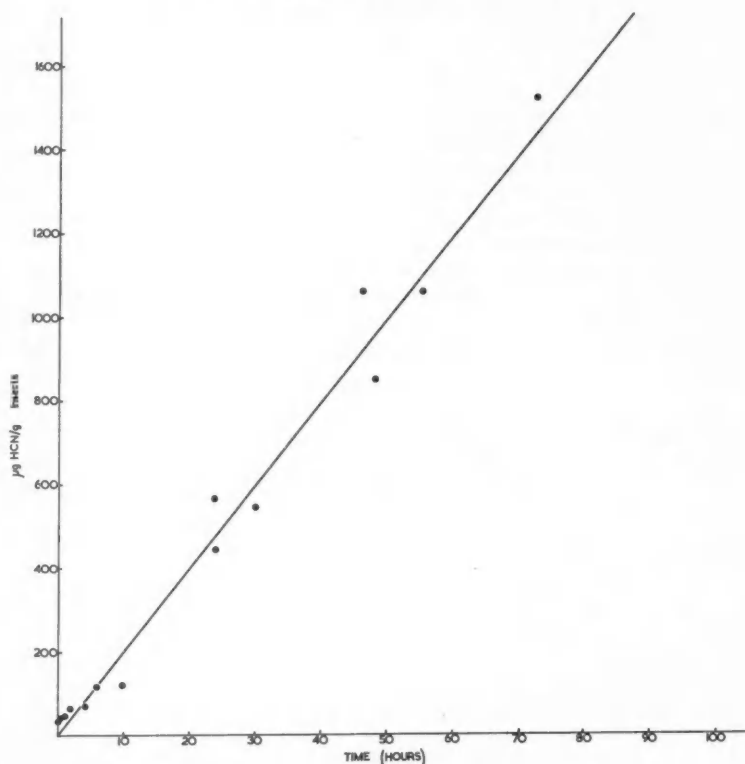


FIG. 4. The amount of HCN recovered from insects exposed to a constant concentration of fumigant (7 mg/l.).

disappearance of hydrogen cyanide coincided with the return of sensitivity and activity, it seemed highly probable that recovery depended on the removal of hydrogen cyanide.

Further experiments using insects that had recovered from treatments showed that 58% of the recoverable hydrogen cyanide disappeared within

TABLE I

Hydrogen cyanide recovered from *S. granarius* adults at various intervals after treatment for 4 hours at the LD<sub>50</sub> level

HCN sorbed (µg/g)	164.2	196.4	177.7
HCN recovered (µg/g)			
0 hours	76.2	106.6	98.5
4	73.0		
8	67.1		
12	60.0		
20	62.8	79.8	74.6
48	61.9	80.4	
96	62.2		

20 hours after exposure. However, after 288 hours a considerable amount of recoverable hydrogen cyanide was still present and exerted no obvious effects on surviving insects. This finding suggested that recoverable fumigant remaining in the bodies of active survivors exerted very little effect on the insects and that death might be attributable either to that fraction of the sorbed fumigant that disappeared during the recovery from paralysis or to the irreversibly combined fraction.

TABLE II

Recoverable hydrogen cyanide present in survivors at different times after exposure to a concentration of 8 mg/l. for 4 hours ( $LD_{50}$ )

Survivors	Batch No.	HCN recovered ( $\mu\text{g/g}$ ) after exposure for:				
		0 hr	20 hr	48 hr	96 hr	288 hr
	A	76.2	55.5	53.3	52.6	—
	B	106.6	52.2	66.8	—	—
	C	98.5	54.2	—	—	—
	D	71.4	—	—	—	20.9

To estimate the relative contribution of recoverable and irrecoverable fumigant towards the mortality of the insects, an analysis of covariance was applied to assess, in terms of variability of hydrogen cyanide content, the mortality resulting from each fraction. This analysis, based on eight identical treatments, indicated that mortality could not be attributed to one fraction more than the other, and therefore, the mortality must depend on the total

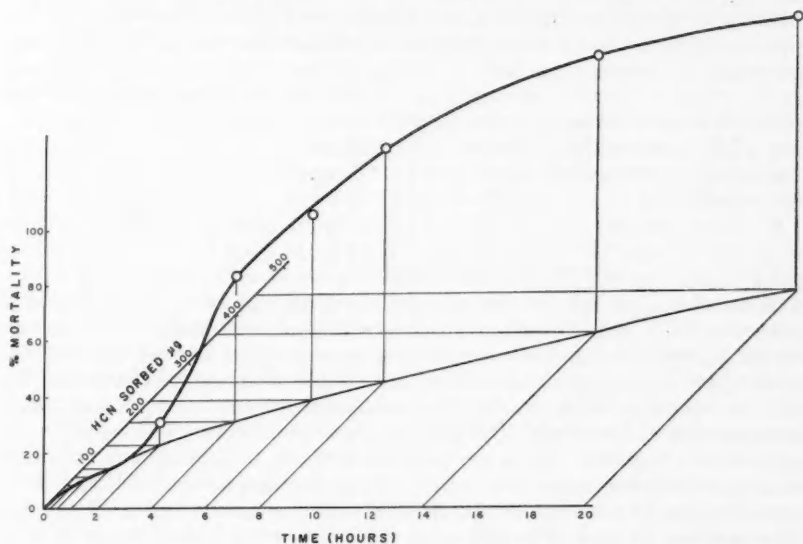


FIG. 5. The relationship of mortality to HCN sorbed by the insects in various periods of time.

amount of fumigant sorbed. The relationship of mortality to the total hydrogen cyanide sorbed in various periods of time is shown graphically in Fig. 5.

The disappearance of reversibly held hydrogen cyanide from insects raised a very important question—the fate of the lost fumigant. Two ways of removing cyanide seemed possible: either the fumigant was being desorbed from the insect and lost to the air, or it was combining irreversibly with the body components. Since aeration of the treated insects failed to show the presence of desorbed hydrogen cyanide the alternative possibility seemed to be the most likely process for removing cyanide. The occurrence of this process during the restoration of sensitivity and activity in the insects would suggest that the cyanide had to be detoxified before normal metabolic activity could be resumed. Two detoxication mechanisms for converting cyanide to less toxic compounds are known to exist in mammals. The enzyme rhodanese converts cyanide in the presence of thiosulphate to thiocyanate and cystine combines with cyanide to form 2-imino-4-thiazolidine carboxylic acid (11). The end products of both of these reactions are less toxic than cyanide, and are excreted in the urine. One of these mechanisms—the detoxication of cyanide using rhodanese—has been suggested as the pathway for detoxifying cyanide in insects (9). *S. granarius* adults were examined for both of these mechanisms.

#### Examination of Insects for Detoxifying Mechanisms

In testing for rhodanese, 10 g of insects was finely ground in 10 ml of ice water, and enzyme extracts were made according to the method outlined by Sumner and Somers (10). To every 10 ml of the brei 2.4 ml of chloroform and 2.4 ml of 95% ethanol were added and the mixture was shaken vigorously for 20 seconds. The material was then centrifuged for 15 minutes at 3500 g and the supernatant liquid was removed and dialyzed overnight against dilute neutral phosphate to remove chloroform and alcohol. To every 10 ml of the dialyzed liquid, 11 ml of neutral saturated ammonium sulphate was added, and the precipitate was removed by centrifuging. Then an equal volume of the ammonium sulphate was added to the remaining liquid to precipitate rhodanese. To this precipitate 0.125 M  $\text{Na}_2\text{S}_2\text{O}_3$  and 0.25% egg albumen were added to make 5 ml of solution.

Rhodanese activity was tested according to the method described in Colowick and Kaplan (3). One milliliter of 0.125 M  $\text{Na}_2\text{S}_2\text{O}_3$ , 0.5 ml of 0.2 M  $\text{KH}_2\text{PO}_4$ , and 5 ml of 0.25 M KCN were mixed in a 50-ml Erlenmeyer flask. One milliliter of enzyme solution was added and the reaction, after being allowed to proceed at 25° C for 15 minutes, was stopped by adding 0.5 ml of 38% formaldehyde. Then 2.5 ml of ferric nitrate reagent was added and the solution was centrifuged to remove suspended solid material. The optical density of the solution was then determined at a wave length of 460 m $\mu$  with a Spekker absorptiometer. Tests with the extract from the insects failed to show the presence of rhodanese. To prove that the enzyme, if present, was not being destroyed or lost at some stage of the concentrating process, fresh pigs' liver, which is known to contain rhodanese, was added to the insect brei during the homogenizing process. The following results, obtained when 0.2 g of liver was ground with 12 g of *S. granarius* adults, showed that rhodanese could be extracted by this method.

EXTRACT	CNS ( $\mu\text{g/ml}$ )
Insects and liver	240
Insects alone	0

One milliliter of this extract diluted 10,000 times and then allowed to react with potassium cyanide and sodium thiosulphate produced 5  $\mu\text{g}$  of thiocyanate in the 15-minute reaction period.

Fumigated insects were also examined for the presence of thiocyanate in their bodies as a further test for the rhodanese detoxication mechanism. One gram of *S. granarius* adults was fumigated with hydrogen cyanide at a concentration of 7 mg/l. for 4 hours, then ground in a mortar with silver sand and 1 ml of water and centrifuged at 3500 g for 15 minutes. Forty  $\mu\text{l}$  of the supernatant liquid was spotted on No. 20 chromatography paper and allowed to ascend this strip in a solvent of *n*-butanol saturated with 10% ammonia for 4 hours. A negative test for thiocyanate resulted when the chromatogram was sprayed with ferric nitrate. By this method 0.1  $\mu\text{g}$  of thiocyanate could be detected. If cyanide had been converted to thiocyanate in the insects up to 265  $\mu\text{g}$  of thiocyanate might have been present in the insects' bodies. Further tests using insect brei fortified with a known amount of thiocyanate showed that thiocyanate could be detected in extracts of the brei and that it was not destroyed in appreciable amounts within 12 hours of preparation. The failure to detect either thiocyanate or rhodanese indicated that this pathway was of no importance in the detoxication of hydrogen cyanide in *S. granarius*.

Similar tests were carried out on nymphs of the desert locust *Schistocerca gregaria* Forsk. This insect sometimes feeds on cyanogenetic plants and might be expected to have a detoxifying enzyme such as rhodanese to remove excess amounts of cyanide from its body. Neither the enzyme nor thiocyanate could be found in fumigated insects.

The other mechanism for detoxifying cyanide, the combination of cyanide with cystine to form 2-imino-4-thiazolidine carboxylic acid, could not be found in *S. granarius*. By using hydrogen cyanide labelled with carbon-14, the labelled metabolic products were separated and shown to be different than 2-imino-4-thiazolidine carboxylic acid.

### Discussion

The linear pattern of uptake of hydrogen cyanide by insects during fumigation suggests that the rate of uptake is governed by the rate of decomposition of hydrogen cyanide as it combines with body components rather than the rate of diffusion to these sites within the body. It is noteworthy that sorption continued at a steady rate long after a lethal amount had been taken up. The recoverable fraction continued to increase steadily for at least 96 hours when more than 8 times the amount required to kill had been sorbed. Other workers (1, 4, 8) have placed some emphasis on the recoverable fumigant, but results presented here show that a large percentage of it in one species of insect exerted no apparent biological effect. However, some of this fraction—that which disappeared as the insects regained sensitivity—may be important in bringing about the death of the insect.

The removal of hydrogen cyanide during the recovery period suggested that it was being detoxified. Since the mechanisms which detoxify cyanide in mammals could not be found in either the granary weevil or the desert locust, some other detoxifying system must have been operating. It seems possible that blockage of the detoxifying system may be closely linked with the lethal effects of the poison.

### Acknowledgments

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## THE ACTION OF FUMIGANTS ON INSECTS

### II. THE EFFECT OF HYDROGEN CYANIDE ON THE ACTIVITY AND RESPIRATION OF CERTAIN INSECTS<sup>1</sup>

E. J. BOND<sup>2</sup>

#### Abstract

Studies have been made on the mode of entry and the effect of hydrogen cyanide on the respiration of insects and have shown that cyanide caused complete respiratory inhibition in *S. granarius* but not in the other insects studied. The response of *S. granarius* to anoxic conditions differed from its reaction to cyanide, and insects paralyzed with cyanide were more resistant to methyl bromide than those which were active. Insects died when the amount of cyanide absorbed, calculated as a molar concentration of their total body water, was about the same as the concentration at which it combines with sugars to form cyanhydrins.

#### Introduction

The respiratory system is the most likely path by which a fumigant can enter an insect's body. Since the spiracles and tracheae are used for natural gas exchange, any response of the insect that affects the respiratory system will also be likely to affect the uptake of fumigant. Previous studies (2) have shown that susceptibility to one fumigant, methyl bromide, is directly associated with the rate of respiration. Hydrogen cyanide is characteristically a respiratory poison that causes rapid paralysis, and both respiratory inhibition and paralysis produce effects on insects that could be important in uptake of the fumigant. For instance, rapid paralysis will decrease oxygen requirements; decreased oxygen requirements should partially compensate for the anoxic effects of the poison. After paralysis, spiracular movements and body pulsations that are normally associated with oxygen uptake will stop and thereby eliminate the assistance that these movements might give in fumigant uptake. To assess the effect of the fumigant on respiratory activity, observations were made on the condition of the spiracles of *Schistocerca gregaria* Forsk. and *Phormia regina* (Meig.) after treatment, and measurements were taken on the oxygen consumption of *Sitophilus granarius* (L.) and *Tenebrio molitor* L. Since respiratory inhibition and histotoxic anoxia are characteristic physiological actions of hydrogen cyanide, the effect of depriving the insects of oxygen by other means was investigated to compare the relative effects. *S. granarius* adults were exposed to an oxygen-free atmosphere of nitrogen for various periods and the effects, on both activity and mortality, were compared with those caused by hydrogen cyanide.

To determine the protective effect of paralysis and respiratory inhibition on fumigant toxicity, *S. granarius* adults were paralyzed by hydrogen cyanide

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and then exposed to methyl bromide. The mortality resulting from this treatment was compared with that from insects exposed to methyl bromide alone. Methyl bromide was chosen for the test because the susceptibility of at least one insect to this fumigant is directly related to the respiratory rate.

### Experimental

#### *The Effects of Hydrogen Cyanide on Activity and Spiracular Movements*

Some insects are rapidly paralyzed by hydrogen cyanide, while others are more resistant to its paralytic effects. The following table shows the time required for various insects to be paralyzed when exposed to a concentration of 8 mg/l. of hydrogen cyanide.

INSECT	PARALYSIS TIME (MINUTES)
<i>S. granarius</i> adult	2
<i>S. gregaria</i> adult	5
<i>P. regina</i> adult	1
<i>P. regina</i> larva	120
<i>T. molitor</i> larva	15

Concomitant with paralysis of the appendages, respiratory movements, which aid in ventilation and convection, also ceased. The pulsating movements of the abdomen of *S. gregaria* which accompany opening and closing of the spiracles stopped, as did the abdominal movements of *T. molitor* larvae and adults. The condition of the spiracular valves after paralysis is likely to be a governing factor in the entry of fumigant to the respiratory system. The position of the spiracular valves of treated *S. gregaria* is shown in Table I. About one-half of the spiracles were completely closed after paralysis while the rest remained completely or partially opened. Spiracle No. 2, the metathoracic spiracle, was always found in an opened position after paralysis while spiracles Nos. 4 and 5 were nearly always closed and the condition of other spiracles was variable. When adults of *P. regina* were exposed to a concentration of 8 mg/l. for 15 minutes, the metathoracic spiracles were found to be completely closed.

TABLE I

The position of the spiracular valves of *S. gregaria* male adults after 15 minutes' exposure to HCN (8 mg/l.)

Spiracle	Valve position	
	Opened	Closed
No. 2	20	0
No. 3	10	10
No. 4	2	18
No. 5	1	19
No. 10	16	4
Total	49	51

#### *The Uptake of Hydrogen Cyanide through the Spiracles*

*T. molitor* larvae were used to investigate the uptake of hydrogen cyanide through the spiracles. The spiracles of fifth instar larvae were sealed by applying beeswax to the spiracles and melting it with a hot wire. Three lots of seven larvae were exposed to a concentration of 8 mg/l. for 4 hours. Another group

TABLE II  
The HCN sorbed by and recovered from *T. molitor* larvae with sealed and unsealed spiracles

	HCN ( $\mu\text{g/g}$ )		
	Sorbed	Recovered	Unrecovered
Spiracles sealed	159.1	116.4	42.7
	155.1	146.5	8.6
	65.9	60.6	5.3
	$126.7 \pm 38.9$	$107.8 \pm 35.6$	$18.9 \pm 16.9$
Spiracles unsealed	411.8	380.1	31.7
	352.3	294.9	57.4
	355.1	317.4	37.7
	$373.1 \pm 27.4$	$330.8 \pm 36.1$	$42.3 \pm 11.0$

of larvae was similarly treated except that the wax was applied above the spiracles without obstructing the openings. The results of these experiments are shown in Table II. It was impossible to ascertain that sealed spiracles were made completely gas-tight and some variation in the uptake may have been attributable to this difficulty. The results do show that total uptake was reduced by 66% by sealing the spiracles and therefore at least this much of the fumigant may enter through the spiracles of this insect.

*The Effect of Hydrogen Cyanide on the Respiration of Insects*

The effect of the fumigant on respiration was determined by fumigating the insects and then measuring their rate of oxygen consumption in a Warburg respirometer. After exposure to an 8 mg/l. concentration of hydrogen cyanide the insects were placed in 20-ml Warburg flasks which contained 0.2 ml of 10% potassium hydroxide for carbon dioxide absorption.

The respiration of *T. molitor* larvae and adults was measured individually while that of *S. granarius* adults was measured collectively (50 insects per flask). The results (Table III) show that oxygen consumption was completely inhibited in *S. granarius* adults after less than 15 minutes' exposure to the fumigant, but that *T. molitor* larvae continued to respire even after 4 hours' exposure. In *T. molitor* adults respiratory inhibition was 84% after 15 minutes'

TABLE III  
Oxygen consumption ( $\mu\text{l/g/hour}$ ) of insects 1 hour after exposure to an 8 mg/l. concentration of HCN

Insect	Time exposed to HCN (minutes)				
	Control	5	15	30	240
<i>S. granarius</i> adult	2400	281	0	—	—
<i>T. molitor</i> adult	874	—	140	—	—
<i>T. molitor</i> larva	573	—	300	174	55

exposure and continued at this level for at least 24 hours; nevertheless, the insects died without recovery from paralysis. *S. granarius* was not killed by the short exposure; respiration was gradually resumed along with recovery from paralysis about 2 hours after treatment (Fig. 1). Oxygen consumption increased steadily until it had reached a rate of 2100  $\mu\text{l}$  per hour in 14 hours—a consumption 30% above that of the untreated insects, and this abnormal rate was reached when 81% of the insects had recovered from paralysis. Although the respiratory rate then began to decline and approach that of the untreated insects, it was still 20% above their rate at 30 hours after exposure.

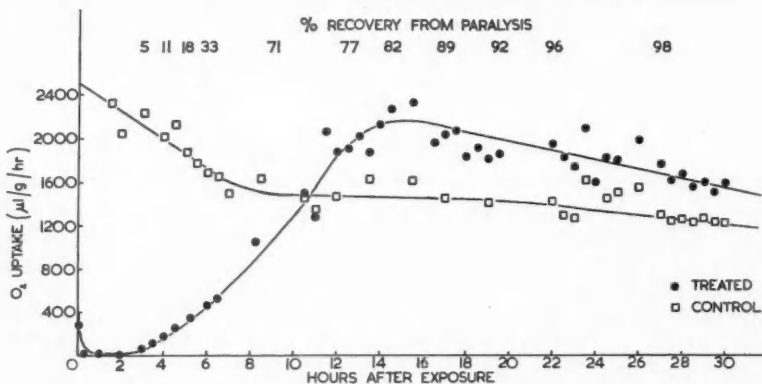


FIG. 1. The effect of a 15-minute exposure to HCN (8 mg/l.) on the respiration of *S. granarius* adults.

#### The Effect of Oxygen Exclusion on *S. granarius* Adults

The insects were placed in a 300-ml air-tight chamber and exposed to an atmosphere of pure nitrogen for various periods of time to determine the effects of anoxia. Oxygen was removed from the chamber containing the insects by evacuating to a pressure of 0.5 mm of mercury and refilling with oxygen-free nitrogen. This process was repeated 4 times to ensure that all oxygen was removed. A small dish of 20% pyrogallol solution in the chamber ensured complete absence of oxygen. Commercial cylinder nitrogen was purified by passing it through a vanadyl sulphate solution as outlined by Meites and Meites (9).

The mortality resulting from exposing the insects to an oxygen-free atmosphere for 72, 84, 98, and 117 hours was 27, 54, 63, and 100% respectively. When the insects were exposed to pure nitrogen they continued to move about for nearly 5 hours; however, activity gradually and progressively decreased until no further movement was evident. It is interesting to note that complete and rapid paralysis took place when the insects were exposed to low pressures during the removal of oxygen but apparently normal activity returned within 2 or 3 minutes of restoring normal pressure by admitting nitrogen. Evidently this paralysis was not the result of tissue anoxia but rather was caused by physical factors induced by low pressures.

*Susceptibility of Insects, Paralyzed by Hydrogen Cyanide, to Methyl Bromide*

*S. granarius* adults were paralyzed by exposure to hydrogen cyanide at a concentration of 8 mg/l. for 15 minutes and then treated with a concentration of 5.5 or 8.0 mg/l. of methyl bromide for 5 hours to determine whether prior paralysis altered their susceptibility to the latter fumigant. The results in terms of mortality were as follows:

	CH <sub>3</sub> Br (mg/l.)					
	5.5			8.0		
	Total	Dead	Mortality (%)	Total	Dead	Mortality (%)
Pretreated with HCN	925	110	12	1080	455	42
Not pretreated	678	384	57	687	687	100

Of the insects exposed to the 5.5 mg/l. concentration 45% were protected by pretreatment with hydrogen cyanide and of those exposed to 8.0 mg/l. methyl bromide 58% survived.

*Changes in Aqueous Extracts after Treatment of S. granarius with Hydrogen Cyanide*

After *S. granarius* adults had been exposed to hydrogen cyanide the consistency of aqueous extracts became noticeably different. Extracts from insects exposed to 7 mg/l. of cyanide for 20 hours could be filtered much more readily than a similar extract from untreated insects. Also when a 25- $\mu$ l sample of the extract was spotted on chromatography paper (in 5- $\mu$ l aliquots) the pigment in the sample from treated insects diffused outwards to the perimeter of the spot whilst pigment in the sample from untreated insects accumulated in concentric bands towards the center. There appeared to be less pigment in the extract from the treated insects than from those that were not treated.

**Discussion**

There is wide variation in the time required for hydrogen cyanide to paralyze different species of insects and different stages of the same species. *P. regina* adults were paralyzed in 45 seconds while the larvae of this species did not become paralyzed before 2 hours of treatment. The difference in resistance between these two insects might be attributable either to their relative ability to exclude fumigant from the site of paralytic action or it might be associated with a difference in resistance of the site of action itself. In *S. granarius*, paralysis did not appear to be directly associated with respiratory inhibition because paralysis from hydrogen cyanide treatment was complete within 2 minutes and preceded respiratory inhibition, whereas muscular activity continued in an oxygen-free atmosphere for more than 5 hours.

The position in which the spiracular valves come to rest in a paralyzed insect will influence the rate of diffusion of the fumigant through the spiracles. However, even complete closure will not exclude fumigant entirely because gases can diffuse through closed spiracles. Observations made on *S. gregaria*

and *P. regina* showed that spiracular condition after paralysis varied with different spiracles. Spiracular movement in insects is largely governed by the surrounding concentrations of oxygen and carbon dioxide (4); both anoxia and accumulation of carbon dioxide cause the spiracular valve to open. Since hydrogen cyanide might produce either of these conditions, anoxia by inhibition of cytochrome oxidase or accumulation of carbon dioxide by inhibition of carbonic anhydrase, the spiracular valves, particularly those associated with inspiration, would be expected to come to rest in an opened position. Hamilton (7) has shown that spiracles 1, 2, 3, 4, and 10 of resting *S. gregaria* are inspiratory in function; these spiracles would then be expected to remain opened under anoxic conditions. However, the response of most of the spiracles on treatment was variable.

The action of hydrogen cyanide on muscles might be expected to provide an explanation for spiracular response. Since it is known that hydrogen cyanide can impair and sometimes completely inhibit muscle contractibility (10), the spiracular muscles of these insects might be expected to relax during fumigation. The condition of spiracles in *S. gregaria* would tend to support this explanation. For instance, the metathoracic spiracle is opened by the elasticity of sclerotized processes and closing is done by a single occlusor muscle that draws the lips together (1). Relaxation of this muscle by the action of hydrogen cyanide would account for the invariably opened condition of this spiracle. The variable condition of the abdominal spiracles, which are controlled by large fan-shaped occlusor muscles and long narrow dilator muscles, may result from the valve coming to rest in its most relaxed position depending on the position of the abdomen at the time of paralysis. However, the closed condition of the metathoracic spiracles of *P. regina* contradicts this explanation because these spiracles are closed by an occlusor muscle and opened by the elasticity of the spiracle itself. Relaxation of this muscle should cause the spiracles to remain opened. Therefore it is difficult to attribute spiracular condition to any particular action of the fumigant or to relate uptake of the fumigant to the spiracular condition.

Experiments on the uptake of fumigant through the spiracles showed that 66% of the fumigant taken up by *T. molitor* larvae entered through the spiracles. Fraenkel and Herford (5) have shown that 20% of the oxygen consumed by this larva may enter through the body wall when the spiracles are rendered functionless. It is quite possible that the remaining 34% of fumigant sorbed by these insects entered through or even combined with components of the body wall.

Hydrogen cyanide is known to inhibit the respiration of almost all aerobic forms of life by combining with heavy metal enzymes and in particular with the oxidized form of cytochrome oxidase. In *T. molitor* respiration was found to be only partly inhibited even after a lethal dose had been received. Similarly cyanide caused the codling moth respiration to be inhibited by 68–78% (6) and the respiration of *Gastrophilus* larvae to be inhibited by 75% (8). The response of *S. granarius* was unusual because its respiration was completely inhibited in 15 minutes and long before a lethal dose had been absorbed; thus it might be assumed that its cytochrome oxidase was completely inhibited.

This respiratory inhibition probably did not have any direct relationship to the lethal action of cyanide because *S. granarius* was shown to be able to survive anoxic conditions for up to 4 days.

Hydrogen cyanide inhibits respiration in a variety of organisms such as sea urchin eggs, yeast, and bacteria at concentrations of  $10^{-5}$  to  $10^{-3}$  M; both paralysis and cessation of respiration in *S. granarius* took place when the concentration was within this range. Although the combination of hydrogen cyanide with the heavy metal enzymes is reversible, irreversible poisoning occurred when insects were exposed for 4 hours. The irreversibly poisoned insects could be distinguished within 20 hours of treatment because they did not remove any of the recoverable fraction. The mortality could not be directly attributed to inhibition of aerobic respiration because these insects could survive 98 hours' exposure to completely anoxic conditions. However, hydrogen cyanide also combines with glycolysis intermediates at a concentration about  $10^{-2}$  M, which is very nearly the same as the concentration calculated to be in the insects when 50% are killed. Complete blockage of carbohydrate metabolism, with the consequent cessation of all energy release by glycolysis, is likely to be far more detrimental to the insect than is the inhibition of aerobic respiration alone. Hydrogen cyanide is also known to activate proteolytic enzymes like cathepsin (3), and this may contribute to its toxic effects on insects. The changes noted in aqueous extracts from fumigated insects may be associated with some such activation.

The combination of hydrogen cyanide with sugars in the insect's body may be an important factor in the resumption of respiration of *S. granarius*. For instance, in experiments on the oxidation of fructose in salt solution, it was observed that hydrogen cyanide at first completely inhibited the reaction but after a few hours the inhibition disappeared (11). Experiments showed that most of the hydrogen cyanide had been removed from the solution by the formation of fructose cyanhydrin. When hydrogen cyanide inhibits an enzymic reaction a dissociating complex with the enzyme results. The combination of hydrogen cyanide with cytochrome oxidase, producing respiratory inhibition, would result in the formation of such a complex. It seems possible that the resumption of respiration in *S. granarius* may be attributed to dissociation of the cyanide-enzyme complex and the progressive removal of cyanide by combination of the ion with sugars in the insect's body. The sugars would then be acting as detoxifying agents.

### Acknowledgments

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## PREFERENCE OF APHIDS FOR EXCISED LEAVES TO WHOLE PLANTS<sup>1</sup>

J. P. MACKINNON<sup>2</sup>

### Abstract

When adults of *Myzus persicae* (Sulz.) were put on healthy and virus-infected plants or on excised leaves of *Physalis floridana* Rydb., they remained longer on the leaves than on the whole plants, and longer on the virus-infected plants or leaves than on comparable healthy ones. The numbers of nymphs found on both plants and leaves, whether virus-infected or not, appeared to be proportional to the numbers of adults and the length of time that these remained on the hosts.

### Introduction

Some species of plants are suitable hosts for *Myzus persicae* (Sulz.) whereas others are not. And even, within a species, different varieties show marked differences in their suitability to this insect (1, 13). Furthermore, infection with virus has also been shown to make certain plants more acceptable to aphids (2, 3).

Earlier I found (9) that *M. persicae* could not be reared satisfactorily on healthy plants of *Physalis floridana* Rydb., but they could be reared on plants of this species infected with turnip latent (TLV) (8) or potato leaf roll virus (LRV). Later, I found that *M. persicae* would colonize on either healthy or virus-infected excised leaves of this host (11). This paper reports a comparison of healthy and virus-infected *P. floridana* plants and excised leaves as hosts for *M. persicae*.

### Materials and Methods

*P. floridana* seedlings in the two-leaf stage were planted singly in small pots. Some of these were later infected with either TLV or LRV by aphids. When the plants were 6 weeks old, a leaf was cut from the middle of each and placed in water in an Erlenmeyer flask—the petiole inserted through a cotton plug. One healthy plant and one infected with each virus, together with the excised leaf from each, were then placed in a screened cage. Five such cages were used in each experiment. Five large apterous aphids or late instar nymphs, selected at random from colonies on rape, were placed on each experimental plant and each excised leaf. The aphid colony on rape was originally started from a single insect. The hosts were examined each day for 7 days thereafter and the numbers of adults and nymphs on each were recorded.

These studies were made from October to January when the natural photoperiod averaged about 8 hours. A 200-watt incandescent bulb, placed over each cage, supplied an additional 8 hours of light. Temperatures ranged from 14 to 18° C at night and 20 to 26° C in the day.

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### Results and Discussion

The results of five experiments are shown in Fig. 1. Adults remained longer on whole plants infected with either virus than on healthy whole plants (Fig. 1A). They also remained longer on excised leaves than on comparable whole plants, and longest of all on leaves infected with LRV. The numbers of nymphs (Fig. 1B) were proportional to the length of time that the adults remained on the hosts; the largest numbers on leaves infected with LRV and the least on healthy plants. Apparently, nymphal losses through death and wandering off the hosts exceeded the numbers born in the last days of the experiments as shown by the downward curves in the graphs.

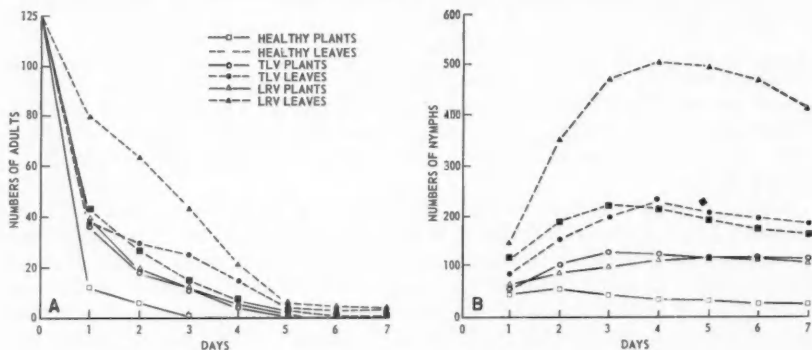


FIG. 1. (A) Time that adults of *M. persicae* remained on *P. floridana* plants and excised leaves in large screened cages. (B) Numbers of nymphs on plants and leaves each day after adults were put on.

Other experiments were done to determine the effect of placing excised leaves in small separate cages where aphids could easily return to the leaves if they fell or wandered off. A middle leaf was taken from each of five healthy, five TLV-infected, and five LRV-infected plants. These were placed in flasks as before and each leaf was covered with a lantern globe. Five aphids were placed on each leaf. The results of three experiments (75 aphids) are given in Fig. 2. Again the adults remained longest (Fig. 2A) and the most nymphs were found (Fig. 2B) on leaves infected with LRV, although in these cages all colonies appeared to do better than had comparable colonies in the large cages.

Further experiments were done to learn if aphids would remain longer on leaves that had been detached from *P. floridana* plants for 48 hours than on newly cut leaves. The leaves were put in flasks under lantern globes as in the experiments just described. In three experiments (75 aphids), there were no differences that could be attributed to time of leaf incision either in the length of time adults remained on the leaves or in the numbers of nymphs found. As in the previous tests, however, adults remained longest and the greatest number of nymphs were found on leaves infected with LRV.

Varying the physiological condition of a plant or part of a plant appears to influence its suitability for aphids. Kennedy *et al.* (5) showed a relation be-

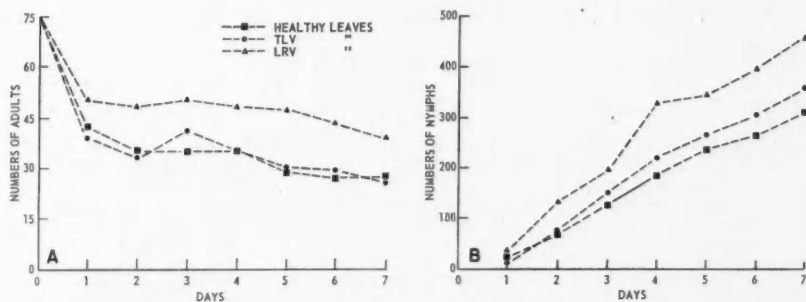


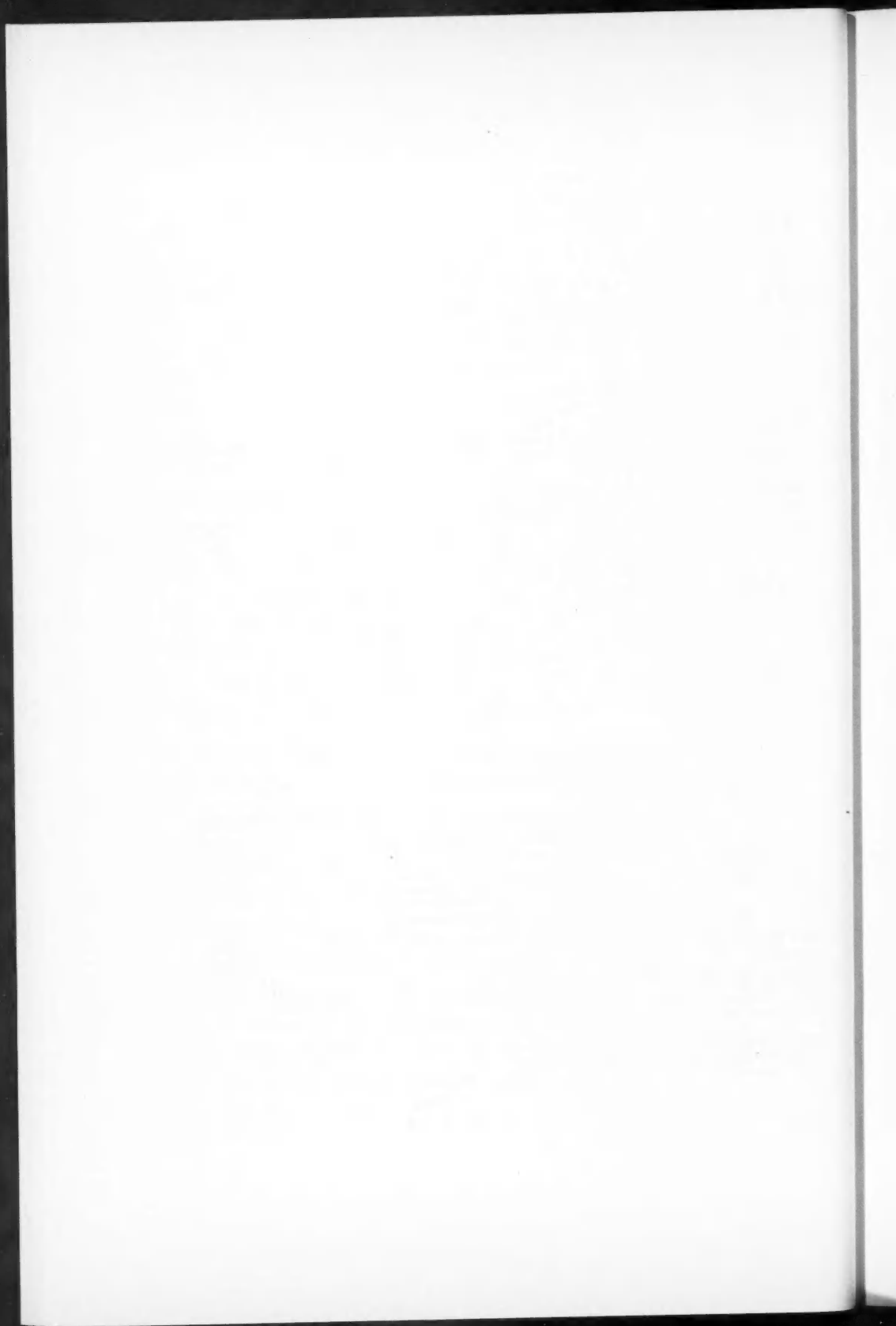
FIG. 2. (A) Time adults of *M. persicae* remained on excised leaves of *P. floridana* in lantern globe cages. (B) Numbers of nymphs on leaves each day after adults were put on.

tween age of leaves and susceptibility to colonization of two aphid species. Also, Kennedy (4) showed a correlation between aphid behavior and plant water relations. MacGillivray (7) reported larger populations of *M. persicae* on excised potato leaves than on whole plants. Excised leaves have also been used successfully as sources of virus for aphids (6, 10, 12).

My results with *M. persicae* on healthy and virus-infected excised leaves are in general agreement with those of other workers so far as populations are concerned. But, I have shown that the use of excised leaves or infection of host plants with viruses did not stimulate adults in producing young. Rather the number of young depended on the time that the adults remained on the hosts.

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# MICROECOLOGICAL FACTORS IN OYSTER EPIZOOTICS<sup>1</sup>

MARSHALL LAIRD<sup>2</sup>

## Abstract

On oyster beds as elsewhere, mud-water interfaces exhibit a biological film harboring saprobic microorganisms. The abundance of these is directly proportional to the amount of decomposing organic matter present. Different communities of protozoa and bacteria characterize each of the levels of organic pollution, which may be defined biologically in terms of a modification of the "Saprobien system" of Kolkwitz and Marsson. While the microorganisms concerned are not conspicuous on clean substrata except where especially favorable conditions are afforded certain of them by some dead animal or plant, their general abundance is indicative of pollution. This follows overaccumulation of reducible deposits, initiated by irregular tidal flushing, freshets, and winter icing, and rendering the microenvironment anaerobic. *Ostrea edulis* and *Crassostrea virginica* survive temporary exposure to the direct effects of such conditions as well as to near-lethal temperatures, but, weakened thereby, become vulnerable to invasion by saprobes from the enriched biological film. Unless environmental conditions improve before the oysters lose ability to recover, abnormal mortalities will be hastened by the activities of these microorganisms, notably *Hexamita inflata* (Protozoa). Relevant literature is reviewed, and possible local remedies are discussed. Canadian data are supported by original observations from Wales and Pakistan. Among other new host and locality records, *Cristispira balbianii* (Spirochaetales) and cercariae of a bucephalid trematode are listed from *Ostrea belcheri* from Karachi.

## Introduction

Mass mortalities of American oysters, *Crassostrea virginica* (Gmelin), occurred on Prince Edward Island in 1915. Since then, "Malpeque disease" (named for the bay where an outbreak was first noticed) has been widely reported from the island (100) as well as from New Brunswick and Nova Scotia (76, 77). The decline in the oyster fishery of the Maritime Provinces has been attributed to this malady, suggested explanations for which rival in number those who have investigated it. Whatever their cause or causes, the gross symptoms of "Malpeque disease" are gaping, thinness, poor shell growth, failure to spawn, and the development of yellow-green pustules (76, 100). Quite commonly, the victim fails to exhibit one or even more of these manifestations.

There appears to be a high degree of contagiousness with the death of up to 99% of the stock, and it has been claimed that resistant strains develop from survivors. Furthermore, suspect seed oysters were brought into Malpeque Bay from the United States a year before the first outbreak (76). These facts might perhaps be accepted as circumstantial evidence for viral causation, which has yet to be disproved. Logie (76) isolated at least two species of bacteria from the blood of mainland *C. virginica*, including some which became sick after transfer to Prince Edward Island. In this connection,

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lesions resembling Needler's pustules had already been reported from Australian oysters (127), and had been thought due to bacteria. Needler (99) concluded that a slime mold (Mycetozoa) was probably responsible, and Richardson (124), the first to record flagellates (*Hexamita* sp.) and ciliates (*Orchitophrya* sp.) from Canadian oysters, also argued for protozoan causation. Interest in hexamitiasis was revived in 1950-51, when the condition was recognized in Holland and the Gulf of Mexico (89). The possibility that this is a specific and highly fatal disease of oysters is currently under investigation at Malpeque Bay (29).

A trial introduction of European oysters (*Ostrea edulis* L.) from Conway, North Wales, to St. Andrews, New Brunswick, was made in the spring of 1957. The consignment was regarded as disease-free. Before replanting, the animals were carefully freed from macroscopic epibionts to avoid accidental pest establishments (78, 95). A month after their arrival they had suffered a 95% mortality, attributed to prolonged air exposure in transit (95). During June, 1958, a visit to the St. Andrews Biological Station of the Fisheries Research Board of Canada provided opportunities for studying survivors of this batch. They proved to be in good condition, and all but seven, the gills of which had become infested with a ciliate normally commensal on clams (p. 455), were free from parasites. However, a second consignment had been imported from the same source by air freight in the spring of 1958. Many of these had become thin and watery, and their examination disclosed ciliates on the mantle and (in one instance) *Hexamita* in the intestine. Several diseased American oysters sent down from the substation at Eglarville, Malpeque Bay, also yielded protozoa, including *Hexamita*.

The European oysters studied had all been held in Sam Orr Pond, a brackish inlet of Passamaquoddy Bay some 60,000 square meters in area located near St. Andrews. Three hundred and fifty of the 1958 batch were now transferred to Eglarville, some being held there in the brackish waters of the Bideford River and others in laboratory tanks. By late February, 1959, all were dead, about two-thirds of those examined harboring *Hexamita* (29). On March 5, 1959, a tray with part of the 1958 consignment was brought up from beneath 0.7 m of ice at Sam Orr Pond, and 98% of the oysters proved to be gaping or dead. Seven were sent to the Institute of Parasitology, but failed to arrive until the afternoon of the 12th. Although decomposing by then, all had huge numbers of active trophozoites of *Hexamita* throughout the tissues and over the body surfaces. The flagellate was duly recorded from all oysters remaining in the tray in question (28, 95).

As it strongly resembled *Hexamita inflata* (p. 469), a well known polysaprobe (74) occurring in polluted brackish (19) as well as fresh waters and long ago recorded from oysters and oyster beds in France (16), mud samples were requested. These were obtained from Sam Orr Pond from beneath 0.65 m of ice on April 7, and proved to support a number of polysaprobic flagellates including undoubted *H. inflata*. Two weeks later, when the last of the ice had melted away, it was found that there were no survivors of either importation. On the other hand, approximately 60% of 200 oysters of the 1958 shipment, held in the laboratory at St. Andrews, were still alive at the end of April (28).

A third consignment from Conway was air-freighted to St. Andrews, via Montreal, in 1959. These oysters arrived on May 2, and 11 of them, after spending a week end in salt-water tanks at the Biological Station, were received at the Institute of Parasitology on May 6. All were still alive, and although two bore a few colorless flagellates (*Monas guttula* and *Rhynchomonas nasula*) on the gills, none harbored any *Hexamita* whatsoever. At the same time, seven survivors of the 1958 shipment, overwintered in the Biological Station laboratory, were received for study. Three were at an advanced stage of decomposition on arrival, and yielded only bacteria. The other four (in one of which ciliated epithelium was no longer active) harbored *H. inflata*, trophozoites being present on the body surfaces and abundant in the digestive gland and blood.

The oysters imported in 1959 were now planted, most of them in Sam Orr Pond. Some were subsequently transferred to Ellerslie while the remainder were established at the Biological Station (both in tanks and at the wharf, below low-tide mark) and in Oak Bay, a few miles west of St. Andrews. Oak Bay was the site of an abortive plant of American oysters in 1896, from which the establishment of *Asterias forbesi* in the Bay of Fundy is said to date (44). Intermittent examinations made by Mr. P. J. Downer up to July 16 revealed no flagellates in either healthy or moribund oysters at Sam Orr Pond. On July 24, however, 25 gapers all proved to be heavily infected with *Hexamita* (28).

A second period was spent at the St. Andrews Biological Station throughout August, 1959. On July 31, only 5 of 13 gapers from Sam Orr Pond had been found positive for *H. inflata*. The apparent decrease in incidence continued, for on August 6 six of eight gapers proved negative, as did four oysters in good condition on the 10th, and two decomposing examples on the 12th. Summer passed without any records of hexamitiasis from Oak Bay or from the oysters kept submerged at the Biological Station wharf. As summer progressed, though, the survivors of the 1958 batch overwintered at the laboratory continued to die, all literally teeming with *H. inflata*. By August 31, only 49 (25% of those placed there early in the preceding winter) remained alive (28).

It had been concluded (28, 95) that the mortalities of 1958-59 among introduced European oysters resulted from prolonged exposure to the low water temperatures of a severe winter. At the same time, it had become abundantly clear that neither the initial failure to establish *O. edulis* in New Brunswick nor epizootics among American oysters could be evaluated properly without clearer appreciation of the role of *Hexamita*. The microbiology of European oysters and their immediate environment at Sam Orr Pond thus received considerable attention during the August, 1959, visit to the Biological Station. Brief visits to Conway, North Wales, and to Karachi, Pakistan, made possible by a World Health Organization assignment on another matter in the following month, and to Ellerslie, Prince Edward Island, in July, 1960, provided additional pertinent information. The data are summarized and analyzed herein, morphological accounts of all the microorganisms except *H. inflata* (p. 469) being held over for further papers.

### Oysters and Other Organisms

Shore invertebrates sedentary for most part of their life are forced into intimate relationships with an astonishing variety of plants and animals. Dollfus (26) lists organisms competing for food with oysters to the disadvantage of the latter, or smothering them, either directly, as in the case of algae of the genera *Cladophora*, *Ulva*, and *Zostera* (125), and the gastropod *Crepidula fornicata* (78), or by causing (as does the common edible mussel, *Mytilus edulis*) the rapid accumulation of silt. Perhaps the oddest relationship between these molluscs and plants concerns the balloon weeds, *Colpomenia* spp., phaeophyceous algae having a vesicular thallus that becomes distended with gas. Thick masses of *Colpomenia* growing upon oysters may drag them from their anchorage to the surface, where winds and currents bear them away (84, 126).

In the three centuries since Auzot (5) drew attention to polychaete infestations of European oysters, a great deal has been written concerning predators, commensals, and parasites of these molluscs. At all stages of their life, enemies prey upon them. Prodigious numbers of their eggs are destroyed by copepods and other plankton feeders. Many young oysters fall victim to crabs, which, on occasion, extract even adults from their shells. A full list of predators would include octopi, fish, and wading birds (26). There is general agreement, though, that starfish of several genera and species are the most widespread and destructive of the animals opening and eating oysters. Polyclad turbellarians stand close to the border line between predation and parasitism in their habits, and sometimes attain local importance (63, 16). Notable among these is *Stylochus inimicus*, often referred to as the "oyster leech", a misnomer that should be abandoned in view of the occurrence of a true leech, *Ostreobdella kakibir*, on *Crassostrea gigas* in Japan (112). Predation is most evident below the low-tide mark (147).

As might be expected, many organisms live as epibionts upon, or bore into, the shells of oysters. These range from filamentous bacteria, boring fungi (*Ostracoblabe implexa*), and various perforating algae (e.g., *Ostreobium queckelti*) (26), to representatives of almost all marine animal phyla: Verrill (139) deals with numerous North American examples, and the most exhaustive study of the *O. edulis* shell fauna to date (69) includes detailed records of Protozoa (Foraminifera, folliculinid ciliates), Porifera (Calcarea, Monaxonida, Cornacuspongida), Coelenterata (Hydrozoa, Scyphozoa, Anthozoa), Platyhelminthes (rhabdocoele turbellarians), Nemertea, Nematoda, Annelida (Polychaeta, Oligochaeta), Arthropoda (Crustacea, Arachnoidea), Mollusca (Loricata, Gastropoda, Lamellibranchiata), Prosopygia (Bryozoa), Echinodermata, and Chordata (Tunicata). Only two members of this formidable array (the boring sponge, *Clione celata*, and the polychaete, *Polydora ciliata*) burrow deeply enough or increase in numbers sufficiently to endanger the host in the Dutch waters where Korringa's studies were conducted, the shell fauna being regarded as primarily adventitious (69). Such organisms are nevertheless always liable to flourish to the oyster's detriment through abnormal biotic disturbances. Thus Kavanagh (64) found Japanese *C. gigas* imported to Louisiana much more adversely affected by *Polydora* than were local American oysters. Again, *Polydora* is liable to destruction through undue

dilution of sea water following freshets. Gross putrefaction within shell blisters caused by the worms may then lead to the host's death (149). The Australian device of raising oyster trays off the bottom to minimize polychaete attack has an opposite effect on the Connecticut coast (79). There, however, the worm is not responsible for serious mortalities, although the numerous shell blisters resulting from heavy infestations render oysters unsightly and less saleable (85).

*Polydora hoplura* and allied species are probably more injurious than *P. ciliata* through their habit of dwelling within the shell valves (69). Also located here are various commensals from microorganisms to higher forms such as the hydroid, *Eugymnanthea ostrearum* (94), and the parasitic crab, *Pinnotheres ostreum* (136). In New Jersey the naked goby, *Gobiosoma boscii*, commonly shelters within empty lamellibranch shells, and there is a report of a 21-mm example of this fish from a 7-cm American oyster, in the right dorsal body wall of which it had excavated a bay where it had lived for at least a year (105).

In lighter vein, before turning to internal commensals and parasites, there are records not only of oysters living as epibionts themselves, upon the carapace of crabs (75), but also of a redoubtable example left overnight on a pantry floor and reputed to have killed mice by closing its shell valves upon them (91)!

Viruses have not been isolated from shellfish, although these harbor a variety of bacteria. Much of the relevant literature concerns the normal intestinal flora (9), or species which happen to be thriving in the surrounding medium (10, 38, 46, 92). Decomposing oysters naturally teem with bacteria (59), and the genus *Serratia* has been indicted as a cause of pinkness (98), a condition for which yeasts are also responsible (58). The alimentary tract harbors representatives of genera common in sea water charged with organic matter (*Spirillum*) and marine ooze (*Saprospira*) (11, 24), and well-known commensal spirochaetes (*Cristispira* spp.) associated with the crystalline style of healthy oysters (17, 24, 32, 110, 113).

No bacteria clearly incriminated as specific pathogens are known from this host. Giard (45) indeed described one (*Myotomus ostrearum*) from European oysters suffering from "Maladie du pied", but Dollfus (26) was not only sceptical as to the bacterial nature of this condition but held its status so doubtful as not to warrant further use of the name. *M. ostrearum* is not mentioned in the Sixth (revised) edition of Bergey's Manual of Determinative Bacteriology (11). Orton (113) quotes Cerruti for the information that during the great oyster mortalities at Taranto, Italy, in 1919-20 no pathogens were recognized among the many bacilli, spirilla, and cocci isolated from sickly specimens, although while the disaster was at its height, "there were on the intestine of the oysters and on the gills millions of a filiform bacterium very long and motionless. . . ." This is strongly suggestive of sewage bacteria. *Sphaerotilus natans* or *S. dichotomus*, and it should be noted that investigations during the 1920-21 mortalities on the British coast resulted in the culturing of the latter species, and a *Vibrio* too, from dying oysters (31, 114). The former organism, however, was not considered a primary cause of death, and as for the *Vibrio*, Orton (114) states that this was fed to sound oysters "which, it would appear, seem to rather enjoy it".

A specific fungal parasite, *Dermocystidium marinum*, occurs in American oysters on the South Atlantic and Gulf coasts of the U.S.A., causing considerable warm-weather mortality in waters of relatively high salinity (123). The gregarine protozoon, *Nematopsis ostrearum* (121), is widespread in the same area and host, but does not appear responsible for unusual mortalities (42, 73). Harmless amoebae (*Vahlkampfia patuxent*) have been recorded from the intestine of *C. virginica* (54), and several saprozoic and bacteria-feeding protozoa have been listed from oysters by authors (16, 17, 102, 113, 129) who did not view them as primarily injurious. As already stated, *Hexamita* has been ranked high among oyster pathogens (89, 124).

*Bucephalus haimeanus* and *B. cuculus* are trematode cercariae parasitizing European and American oysters respectively (55). Bucephalids have also been recorded from *Ostrea commercialis* and *O. angasi* in Australia (128). *B. cuculus* occurs in areas of low salinity, and has been reported from New Jersey to Texas. Heavy infections cause parasitic castration of the host, although this has been declared gastronomically beneficial, the oysters concerned remaining fat and of excellent flavor all summer (96). Menzel and Hopkins (96), in fact, record their personal preference for the taste of *Bucephalus* sporocysts over that of oysters! Other trematodes from these molluscs include *Proctoeces ostrea* from the genital gland of *C. gigas* (37), and three further species, together with three nematodes and the pearl-stimulating cestode (132, 133) from a distant relative, the pearl oyster (*Margaritifera vulgaris*) in Ceylon.

Common mussel copepods of the genus *Mytilicola* sometimes spread to *Ostrea edulis* in European waters (8) and to *O. lurida* and *C. gigas* on the west coast of North America (111), without appearing to do much harm to the new host. Oyster parasites reported from other animals in nature or transmitted to them in the laboratory include *Dermocystidium marinum*—for which the quahaug, *Mercenaria mercenaria*, and the soft-shell clam, *Mya arenaria*, serve, albeit poorly, as experimental hosts (123)—and *Bucephalus haimeanus*, which may also infect cockles (55). As might be expected, members of the shell fauna frequently settle on other sedentary animals.

Further information concerning the predators, epibionts, and endobionts of oysters may be had from Dollfus (26), Korringa (69), and the bibliographies of Baughman (6) and Ranson (122). Enough has been said to sketch the complexity of the interrelationships between oysters and other occupants of their biotope, and to serve as a background to the following sections.

### Methods

Most of the organisms listed were first studied alive by phase contrast. Methylene blue was used for vital staining, and nigrosin for dry relief slides. Smears for iron haematoxylin staining were made on albuminized  $\frac{3}{4}$  in. square cover slips (No. 1), and fixed in Davis' modification of Worchester's fluid (75 cc saturated solution mercuric chloride, 20 cc formaldehyde 40%, 5 cc glacial acetic acid). Sections were cut at  $7\ \mu$  from Bouin-fixed material, and stained with haematoxylin/eosin. A phase contrast microscope was also used at Conway, where, as at Karachi a week later, bright field observations were made with a pocket-sized "McArthur" microscope having an attached battery-

powered illuminant. During these visits, cover slip smears made and fixed as above were stored in iodized alcohol in 2 in. by  $\frac{1}{2}$  in. plastic tubes pending my return home.

### Materials

*Cristispira balbianii* (Certes) was found in European oysters at St. Andrews and Conway, in American oysters at Ellerslie, and in *Ostrea belcheri* at Karachi. The only other widely reported specific associates of oysters discovered were bucephalid trematodes. *Bucephalus cuculus* McCrady was recorded from one (3%) of the 30 American oysters dissected, while unidentified bucephalid cercariae were found in three (15%) of the 20 *O. belcheri* examined at Karachi.

The Ellerslie oyster parasitized by *B. cuculus*, and a second example, had astomatous ciliates in the digestive gland. These were referable to *Orchitophrya*, a genus already recorded from Prince Edward Island oysters by Richardson (124), and were morphologically identical with *O. stellarum* Cépède. This species causes parasitic castration of starfish (15, 141), and was first reported from a North American host (*Asterias forbesi*, at Milford, Connecticut) in 1935 (3). Its incidence on Canadian Atlantic shores, where the cold-water purple star (*Asterias vulgaris*) predominates, is unknown.\*

A second ciliate, the thigmotrich *Ancistrocoma pelseneeri* Chatton and Lwoff, was present on the gills of seven (20%) of 35 European oysters of the 1957 importation, when these were taken from Sam Orr Pond in June, 1958. This commensal of soft-shell clams and several other lamellibranchs (70) has not been reported from oysters before.

It was not possible to identify free-living bacteria other than *Spirillum* and *Sphaerotilus*, as culture facilities were not available. Algal determinations were not made either. Other primarily free-living microorganisms recorded from oysters, their shells, and the surrounding mud are listed in Table I. Tables II and III show associations of such organisms, commensals, and parasites with healthy, gaping, and dead European and American oysters.

### Discussion

#### *Obligate and Facultative Parasites*

*Cristispira balbianii* is a commensal, the absence, not the presence, of which is a sign of unhealthiness of the host. First described from European oysters (16, 17), and later from *C. virginica* (101, 110), it is now listed for the first time from *Ostrea belcheri* and Pakistan. It normally occurs in the crystalline style, which soon breaks down after the host is removed from the water or if it becomes sickly (113). The spirochaetes then spread throughout the gut contents and over the gills and mantle (113). Style reformation does not take place under anaerobic conditions (Berkeley, quoted by Nelson (104)), during which *Cristispira* occurs in very small numbers, if at all. In the present studies it was never found in gaping oysters lacking a style and under invasion by saprozoic organisms. Perhaps amylolytic ferments produced by a normal style (104, 106) preclude such invasion of the healthy animal?

\*Since this went to press my attention has been drawn to a research note by G. F. M. Smith (Science, 84, 157 (1936)) recording *O. stellarum* from about 25% of a small collection of female *A. vulgaris* from Malpeque Bay oyster beds.

TABLE I  
Normally free-living organisms recorded from oysters and their immediate vicinity  
(C = common, R = rare, X = present, incidence indicated in Tables II and III)

(Relevant references follow each name in parentheses)	<i>O. edulis</i>			<i>C. virginica</i>			<i>O. belcheri</i>		
	St. Andrews, N.B.			Conway, N. Wales			Ellerslie, P.E.I.		
	In mud nearby	On shell	In or on oyster	On shell	In or on oyster	On shell	On shell	In or on oyster	In or on oyster
Eubacteriales									
<i>Spirillum</i> sp.	C	C	X	—	—	C	—	X	—
Chlamydo bacteriales									
<i>Sphaerotilus dicholomus</i> (Cohn) (114)	C	C	X	—	—	—	—	—	—
Mastigophora									
Euglenoidina									
<i>Autoceros acinus</i> Dujardin (19, 47, 71, 142, 148)	R	R	—	R	—	C	—	—	—
<i>Autoceros</i> sp. (71)	R	R	—	R	—	—	—	—	—
<i>Paramecium trichophorum</i> (Ehrenberg) (19, 71, 131)	—	—	—	R	—	—	—	—	—
<i>Mariprognaster picta</i> Faria et al. (33, 148)	—	—	—	C	—	—	C	—	—
<i>Trophidocyphus octocostatus</i> Stein (14, 33, 47, 71, 142)	—	—	—	R	—	—	—	—	—
Dinoflagellata									
<i>Glenodinium foliaceum</i> Stein (19)	R	—	X	—	—	—	—	—	—
<i>Proocentrum micans</i> Ehrenberg (19, 23, 71, 131)	R	—	X	—	—	—	—	—	—
Proromonadina									
<i>Bodo edax</i> Klebs (19)	C	C	X	—	—	C	—	X	—
<i>Bodo rostratus</i> (Kent) (19, 65, 131, 142)	C	C	X	—	—	C	—	X	—
<i>Monas guttula</i> Ehrenberg (33, 47)	C	C	X	—	—	C	—	X	—
<i>Monas minima</i> Meyer (19)	C	C	X	—	—	C	—	X	—
<i>Okomonas termo</i> (Ehrenberg) (19, 71, 140)	C	C	X	—	—	C	—	—	—
<i>Rhynchomonas nasuta</i> (Stokes) (33, 47, 90)	C	C	X	—	—	—	—	—	—
Polynastigina									
<i>Dallia dasydactylus</i> Kent (65, 71)	C	R	X	—	—	R	—	X	—
<i>Hexamita inflata</i> Dujardin (16, 37, 19)	R	R	X	—	—	—	—	—	—
<i>Trepomonas agilis</i> Dujardin (19, 71)	—	—	—	—	—	—	—	—	—
Sarcodina									
Amoebozoa									
<i>Amoeba radiosa</i> Ehrenberg (131, 148)	C	C	X	R	—	—	—	—	—

TABLE I (Continued)  
Normally free-living organisms recorded from oysters and their immediate vicinity  
(C = common, R = rare, X = present, incidence indicated in Tables II and III)

	Microorganism (Relevant references follow each name in parentheses)	<i>O. edulis</i>			<i>C. virginica</i>			<i>O. belcheri</i>	
		St. Andrews, N.B.		Conway, N. Wales	Ellerslie, P.E.I.		In or on oyster	On shell	In or on oyster
		In mud nearby	On shell	In or on oyster	On shell	In or on oyster			
Ciliata									
Holotricha									
	<i>Chilodonella cucullulus</i> (O. F. Müller) (14, 71)	R	C	X	R	—	—	—	—
	<i>Cinetochilum margaritaceum</i> Perty (62, 71)	—	—	X	—	—	—	—	—
	<i>Cinetolabus vermicus</i> (O. F. Müller) (14, 33, 62, 71, 143)	R	C	X	C	X	X	—	—
	<i>Colpidium campylum</i> (Stokes) (62)	—	—	X	—	—	—	—	—
	<i>Colpidium colpoda</i> (Ehrenberg) (14, 113, 143)	—	—	X	—	—	—	—	—
	<i>Cyclidium glaucoma</i> O. F. Müller (33, 71, 74)	C	C	X	C	X	X	—	—
	<i>Eucleocydon</i> sp. (14, 71)	—	—	X	—	—	—	—	—
	<i>Favosites</i> sp. (14, 71)	—	—	—	R	—	—	—	—
	<i>Lucymeria coronata</i> Claparède and Lachmann (67, 148)	R	—	—	—	—	—	—	—
	<i>Lionulus fasciola</i> (Ehrenberg) (14, 33, 62, 71, 74, 143, 148)	R	R	X	—	—	—	—	—
	<i>Paramacium</i> sp. (71)	R	R	—	R	—	—	—	—
	<i>Platyemadum sociale</i> (Penard) (62)	C	C	—	—	—	—	—	—
	<i>Platyemadum maritimum</i> (Dujardin) (14, 71, 148)	—	—	X	C	X	X	—	—
	<i>Uronema maritimum</i> (Ehrenberg) (62, 71, 74)	—	—	X	R	X	X	—	—
	<i>Uronema turbo</i> (O. F. Müller) (62, 71, 74)	R	R	X	R	X	X	—	—
	<i>Uronema</i> sp. (14, 71)	R	R	X	—	—	—	—	—
	<i>Uronema thurcaudum</i> Noland	R	R	X	—	—	—	—	—
	Unidentified tetrahymenids (72, 113)	—	—	—	—	—	—	—	—
Spirotricha									
	<i>Aspidisca costata</i> (Dujardin) (62, 71, 74)	R	R	X	C	X	—	—	—
	<i>Condylostoma galens</i> (O. F. Müller) (14, 33, 62, 71, 148)	—	—	—	—	—	—	—	—
	<i>Eudis charon</i> (O. F. Müller) (1, 33, 62, 74, 114, 148)	R	C	X	R	X	—	—	—
	<i>Foliculina</i> sp. (2, 69)	—	—	—	—	—	—	—	—
	<i>Holosticha kessleri</i> (Wrzesniowski) (14, 62, 71, 148)	—	R	X	—	—	—	—	—
	<i>Keronopsis rubra</i> (Ehrenberg) (62, 71, 130, 148)	—	R	X	—	—	—	—	—
	<i>Uronychia setigera</i> Calkins (14, 71)	—	R	—	—	—	—	—	—
	<i>Uronychia transfiga</i> (O. F. Müller) (33, 71, 143, 148)	—	—	—	—	—	—	—	—
	<i>Uronychia</i> sp.	—	—	—	—	—	—	—	—
	<i>Urostyla gracilis pallida</i> Entz (62)	R	—	X	R	X	—	—	—
Peritricha									
	<i>Zoodamium</i> sp. (71)	—	—	—	R	—	—	—	—

TABLE I (Concluded)  
Normally free-living organisms recorded from oysters and their immediate vicinity  
(C = common, R = rare, X = present, incidence indicated in Tables II and III)

Microorganism (Relevant references follow each name in parentheses)	<i>O. edulis</i>			<i>C. virginica</i>			<i>O. belcheri</i>		
	St. Andrews, N.B.		Conway, N. Wales	Ellerslie, P.E.I.		Karachi, Pakistan			
	In mud nearby	On shell	In or on oyster	On shell	In or on oyster	On shell	In or on oyster		
Suctorina									
<i>Acinetia tuberosa foetida</i> Maupas (120, 143)	—	R	X	—	—	—	—		
<i>Sphaerophrya solitiiformis</i> Lauterborn (74)	—	—	—	—	R	—	—		
gen. et sp. incert.	R	C	X	—	C	X	—		
			Gastrotetracha						
			Rotatoria						
<i>Diplax</i> sp.	—	—	X	—	C	X	—		
gen. et sp. incert.	C	C	X	—	C	—	—		
			Nematoda						

TABLE II  
Microorganisms recorded from the body surfaces and interior of *Ostrea edulis* (all sources)  
(C = common, R = rare)

Microorganism	Healthy oysters		Gapers		Dead oysters	
	Surfaces	Interior	Surfaces	Interior	Surfaces	Interior
Bacteria						
<i>Spirillum</i> sp.	—	R	C	C	C	C
<i>Sphaerotilus dichotomus</i>	—	—	C	R	C	—
<i>Cristispira balbianii</i>	—	C	C	R	—	—
Protozoa						
Mastigophora						
<i>Bodo edax</i>	—	—	R	—	—	—
<i>Bodo rostratus</i>	—	—	R	R	—	C
<i>Dallingeria drysdali</i>	—	—	R	R	C	C
<i>Glennodinium foliaceum</i>	—	C	—	C	—	C
<i>Hexamita inflata</i>	—	—	R	—	C	—
<i>Monas guttula</i>	R	—	—	—	—	R
<i>Monas minima</i>	—	—	C	—	—	—
<i>Prorocentrum micans</i>	—	C	—	—	—	—
<i>Rhynchomonas nasuta</i>	—	—	—	R	—	—
<i>Trepomonas agilis</i>	R	—	C	—	—	—
Sarcodina						
<i>Amoeba radiosa</i>	C	—	—	—	—	—

TABLE II (Concluded)  
Microorganisms recorded from the body surfaces and interior of *Ostrea edulis* (all sources)  
(C = common, R = rare)

Microorganism	Healthy oysters		Gapers		Dead oysters	
	Surfaces	Interior	Surfaces	Interior	Surfaces	Interior
<b>Ciliata</b>						
<i>Ankistrocoma pelseneeri</i>	R	—	—	R	—	—
<i>Aspidisca costata</i>	—	—	C	R	—	—
<i>Chilonella cucullulus</i>	—	—	R	R	—	—
<i>Cinetochium margaritaceum</i>	—	—	C	C	C	C
<i>Coincimbium vermicus</i>	—	—	C	C	—	—
<i>Colpidium campyllum</i>	—	—	C	C	—	—
<i>Colpidium colpoda</i>	—	—	C	C	—	—
<i>Cyclidium glaucoma</i>	—	R	C	R	R	R
<i>Enchelyodon</i> sp.	R	—	C	C	C	C
<i>Euploes charon</i>	—	—	C	R	—	—
<i>Haloticha kessleri</i>	—	—	C	C	—	—
<i>Lionotus fasciola</i>	—	—	C	C	R	C
<i>Pleuronema marinum</i>	—	—	C	C	C	C
<i>Uronema marinum</i>	—	—	C	R	—	—
<i>Urostyla gracilis pallida</i>	—	—	R	C	C	C
Unidentified tetrahymenids	—	—	C	C	—	—
<b>Suctorina</b>						
<i>Actina tuberosa foetida</i>	—	—	C	—	R	R
<b>Gastrotricha</b>						
gen. et sp. incert.	—	—	C	—	C	R
<b>Rotatoria</b>						
<i>Diplax</i> sp.	—	—	—	—	R	—
<b>Nematoda</b>						
gen. et sp. incert.	—	—	R	—	R	R

TABLE III  
Microorganisms recorded from the body surfaces and interior of *Crassostrea virginica* (C = common, R = rare)

Microorganism	Healthy oysters		Gapers		Dead oysters	
	Surfaces	Interior	Surfaces	Interior	Surfaces	Interior
Bacteria						
<i>Spirillum</i> sp.	—	R	C	C	C	C
<i>Cristispira balbianii</i>	—	C	C	R	—	—
Protozoa						
Mastigophora						
<i>Bodo edax</i>	—	—	C	—	—	—
<i>Bodo rostratus</i>	—	—	R	C	C	C
<i>Dallingeria drysdali</i>	—	—	R	C	C	C
<i>Hexamita inflata</i>	—	—	C	R	C	—
<i>Monas minima</i>	—	—	C	R	C	—
Ciliata						
<i>Aspidisca costata</i>	—	—	C	—	—	—
<i>Cohniembus vermicus</i>	—	—	C	C	C	C
<i>Colpidium campylum</i>	—	—	C	C	C	C
<i>Cyclidium glaucoma</i>	—	—	C	C	C	C
<i>Enchelyodon</i> sp.	—	—	C	C	C	C
<i>Euplates charon</i>	—	—	C	C	C	C
<i>Orchilophrya stellarum</i>	—	—	C	C	C	C
<i>Platynematum sociale</i>	—	—	C	C	C	C
<i>Pleuronema marinum</i>	—	—	C	C	C	C
<i>Urocentrum turbo</i>	—	—	C	C	C	C
<i>Uronema marinum</i>	—	—	C	C	C	C
<i>Uronychia transfuga</i>	—	—	C	C	C	C
<i>Urostyla gracilis pallida</i>	—	—	C	C	C	C
Unidentified tetrahymenids	—	—	C	C	C	C
Gastrotricha						
gen. et sp. incert.	—	—	C	—	R	R
Rotatoria						
<i>Diplox</i> sp.	—	—	R	—	R	—
Trematoda						
<i>Bucephalus cuculus</i>	—	—	—	R	—	—

The Ellerslie record (July 7, 1958) for *Bucephalus cuculus* is the most northerly one thus far for this trematode, and the first one for Canada. *O. belcheri* and Karachi constitute new host and locality records for bucephalid cercariae, and the life history of the Pakistan trematode merits local investigation.

Experience elsewhere does not suggest that *Bucephalus* is linked with Prince Edward Island's epizootics. Neither is the presence of *Ancistrocoma pelseneeri* on some European oysters regarded as pertinent to the mortalities at Sam Orr Pond. The usual host, *Mya arenaria*, occurs there, and a tendency to disperse to other molluscs sharing the biotope (70) is in accord with the habits of this ciliate, which has never been regarded as injurious. Of greater significance is the identification of *Orchitophrya stellarum* from two Malpeque Bay oysters. While the observed incidence (7%) was low, both infections of the alimentary tract were heavy and invasion of the intestinal epithelium had taken place. These facts are in accord with Richardson's (124) findings, and it should be noted that incidence of the parasite among male starfish in Connecticut (3) is of similar magnitude (approximately 9%). Up to 22% incidence has been reported from Long Island Sound (52), and spring surges touch 28% in England, where a high level of parasitaemia has been directly related to good feeding (141). The epidemiology of *Orchitophrya stellarum* infections requires further investigation in the light of these data, with attention to the possibilities that the organism is a regular and perhaps harmful parasite of oysters, and that starfish derive their infections from these animals.

Several of the characteristically free-living organisms mentioned in Table I, or closely allied ones, have already been recorded from oysters. Unfortunately, many investigators have disregarded microscopic animals. Sewage bacteria have been mentioned earlier, and Hornell's reference to a fungus-like organism from oysters dying in the 1877-78 epizootic at Arcachon (56) is also suggestive of *Sphaerotilus* or related forms. In addition to *H. inflata*, Certes\* (16, 17) found *Prorocentrum micans* and *Enchelyodon* sp. in the water of *O. edulis*, and sketched two ciliates which cannot be identified with certainty. *P. micans* is commonly ingested by oysters and other lamellibranchs (36, 93, 117). However, water blooms resulting from huge concentrations of this dinoflagellate are a possible cause of abortion in oysters and other larviparous bivalves (78). Nelson (102) illustrated (his Plate IV) several organisms which he observed developing rapidly in small containers in hot weather, when decomposing spat furnished them with suitable food. His Figs. 2 and 3 show a uninucleate ciliate (not *Amphileptus* as he thought probable, members of this genus being binucleate) which might well be *Cohnilembus verminus*, a common meso- to poly-saprobic marine ciliate (62). He also illustrated gastrotrichs (Figs. 4, a-c) and the subject of his Fig. 6 was probably the predacious ciliate, *Lacrymaria coronata*. Ciliates on Nelson's Plate V, "Microscopic parasites and enemies of oysters", include Spirotricha close to *Euplotes charon* (Fig. 12, a, b) and *Aspidisca* sp. (Fig. 13). Orton (113) pointed out that ciliates are very common in weakening oysters, their numbers increasing enormously as the death of the host approaches. He found a suctorian (*Acineta*

\*His results were contemporaneously reported in the United States by Ryder (129).

*tuberosa foetida*), common in decaying seaweed (143), in a maceration of oyster heart in sea water (120), and reported that dying European oysters yielded *Colpidium colpoda*, *Glaucoma scintillans*, and (*Loxophyllum*) = *Lionotus lamella*, besides *Aspidisca* sp. and a *Uronychia*-like organism. However, Orton concluded that all these protozoa were merely secondary invaders equally liable to attack any other weakly or dying marine animal.

It is submitted that the obvious logicity of this conclusion blinded Orton to the real significance of his microorganisms as biological indicators of pollution, a significance that cannot be properly appreciated until they are considered as units and groups in a modified version of the "Saprobiensystem" of Kolkwitz and Marsson (67, 74, 135).

#### *The Microbiology of Polluted Waters*

The Saprobiensystem, as originally conceived and subsequently expanded by its authors (67), listed numerous species of plants and animals, most of them microorganisms, considered characteristic of the different levels of organic pollution. Three zones of pollution were defined, oligosaprobic, mesosaprobic, and polysaprobic. Under the first, the oxidation of polluting substances is all but complete and vigorous disintegration processes have died away. Mesosaprobic conditions prevail during most of the period of oxidation. Under  $\beta$ -mesosaprobic conditions the ammoniacal compounds of the fatty acids are evident, and the fouler  $\alpha$ -mesosaprobic zone is marked by the prevalence of amino acids. Polysaprobic conditions, dominated by the abundance of complex organic matter, are those of heavy pollution and decomposition, with reduction of the polluting substances proceeding vigorously. While this is the classification now adopted, it should be mentioned that certain modern investigators (135) prefer to subdivide the polysaprobic zone too.

Liebmann (74) revised the Saprobiensystem, with greater attention to the life requirements and abundance of the chosen indicator species. Studies of protozoan communities by Picken (118), Fauré-Frémiet (34), and others have also stressed the importance of a broad ecological approach. Recent applications of the system (135) characterize the different levels of pollution by indicator communities of dominant organisms instead of by long lists of species which more often than not tolerate more than one zone. The author (72) has summarized earlier research on the succession of communities of microorganisms as the level of organic pollution changes, and Bick (7) provides quantitative data on such successions.

By its nature less precise than chemical and physical procedures used in water analysis, and primarily intended for application to organically rich stretches of streams, the Saprobiensystem nevertheless lends itself to studies of all kinds of water bodies. Biological balances involve the interplay of so great an array of factors, and tend to be so briefly static, that bioassay methods, used with proper discretion, can form valuable tools for the ecologist. As long as the emphasis is on communities and due attention is given to successions, the Saprobiensystem provides a valid general estimate of organic pollution, and not necessarily of faecal pollution, although it has seen most use in this regard. The microorganisms of biological flocs due to sewage are equally characteristic of films at any mud-water interface in natural waters. They

concentrate in the porous upper part of the film (71), which varies in thickness according to the rate of accumulation of dead plants and animals and other organic wastes not necessarily linked with human activities. Their communities, classified arbitrarily in accordance with the Saprobiensystem, provide a measure of the degree of micropollution. While highly relevant to the well-being of lamellibranchs, this aspect of the bottom microenvironment is liable to escape detection altogether where estimates of pollution are based upon chemical analysis of the overlying water mass.

TABLE IV  
Status of species in the Saprobiensystem

Microorganism	Zone of occurrence			
	Oligo-saprobic	$\beta$ -Meso-saprobic	$\alpha$ -Meso-saprobic	Poly-saprobic
<i>Glenodinium foliaceum</i>	X			
<i>Prorocentrum micans</i>	X			
<i>Amoeba radiosa</i>	X	X		
<i>Anisonema acinus</i>	X	X		
<i>Condylostoma patens</i>	X	X		
<i>Keronopsis rubra</i>	X	X		
<i>Lacrymaria coronata</i>	X	X		
<i>Marsupiogaster picta</i>	X	X		
<i>Uronema pluricaudatum</i>	X	X		
<i>Uronychia setigera</i>	X	X		
<i>Uronychia transfuga</i>	X	X		
<i>Holosticha kessleri</i>	X	X	X	
<i>Monas guttula</i>	X	X	X	
<i>Peranema trichophorum</i>	X	X	X	
<i>Tropidocyphus octocostatus</i>	X	X	X	
<i>Urocentrum turbo</i>	X	X	X	
<i>Aspidisca costata</i>		X	X	
<i>Bodo edax</i>		X	X	
<i>Frontonia marina</i>		X	X	
<i>Lionotus fasciola</i>		X	X	
<i>Platynematum sociale</i>		X	X	
<i>Urostyla gracilis pallida</i>		X	X	
<i>Acineta tuberosa foetida</i>		X	X	X
<i>Chilodonella cucullulus</i>		X	X	X
<i>Cinetochilum margaritaceum</i>		X	X	X
<i>Cohnilembus verminus</i>		X	X	X
<i>Colpidium campylum</i>		X	X	X
<i>Cyclidium glaucoma</i>		X	X	X
<i>Euploes charon</i>		X	X	X
<i>Oikomonas termo</i>		X	X	X
<i>Pleuronema marinum</i>		X	X	X
<i>Rhynchomonas nasuta</i>		X	X	X
<i>Sphaerotilus dichotomus</i>		X	X	X
<i>Uronema marinum</i>		X	X	X
<i>Bodo rostratus</i>			X	X
<i>Colpidium colpoda</i>			X	X
<i>Dallingeria drysdali</i>			X	X
<i>Hexamita inflata</i>			X	X
<i>Monas minima</i>			X	X
<i>Trepomonas agilis</i>			X	X
<i>Sphaerophrya solitiformis</i>			X	X

*The Saprobiensystem in Brackish Environments*

Table IV places the species listed in Table I in the Saprobiensystem, which has seen little use in brackish water biology. Data supporting the necessarily arbitrary allocation of individual microorganisms to the four major zones of pollution are to be found in the references included in the latter table. At the higher levels of pollution there is remarkably close correspondence with freshwater conditions, for most of the characteristic polysaprobic species are perfectly tolerant of a brackish environment. Among the few that are not is *Colpoda cucullus* O. F. Müller, perhaps the most conspicuous omission from these pages, this species being unable to withstand even the most gradual transition to 10% sea water (35). A familiar  $\beta$ -mesosaprobic ciliate, *Halteria grandinella* (O. F. Müller), is absent too. Although Wailes (143) listed this species from brackish pools on Canada's Pacific coast, Finley (35) declared that *Halteria*, like *Colpoda*, is quite intolerant of even slight salinities. At the other end of the scale, some species, like *Oikomonas termo* (50), can be transferred directly to pure sea water provided that their nutritional requirements are met.

Thus all seven species entered only in the  $\alpha$ -mesosaprobic and polysaprobic columns also occur in polluted fresh water or animal macerations. Furthermore, only three (25%) of the saprophiles tolerating polysaprobic through  $\beta$ -mesosaprobic conditions (*Acineta tuberosa foetida*, *Cohnilembus verminus*, and *Pleuronema marinum*) are restricted to brackish waters or the sea; as are three (27%) of the species not found under conditions fouler than  $\alpha$ -mesosaprobic (*Frontonia marina*, *Holosticha kessleri*, and *Urostyla gracilis pallida*). The proportion of marine and brackish water species is much higher, though, in the remaining zones. Five (56%) of the oligosaprobic to  $\beta$ -mesosaprobic species (*Condylostoma patens*, *Keronopsis rubra*, *Uronema pluricaudatum*, *Uronychia setigera*, and *U. transfuga*) are limited to the sea or brackish waters, as is one of the two oligosaprobic dinoflagellates, *Prorocentrum micans*.

*Significance of Association between Oysters and Normally Free-living Microorganisms*

As is evident from Tables I and IV, a single polysaprobic species was recorded from the very thin film of mud on the shells of European oysters at Conway and *Ostrea belcheri* at Karachi. This was *Euplotes charon*, a hypotrichous ciliate found throughout the mesosaprobic zones as well, and known as a facultative parasite of marine invertebrates (sea anemones and echinoderms) (1). A  $\beta$ -mesosaprobic heterotrich, *Condylostoma patens*, present on the valves of oysters at Conway (Table I), has also been reported as a harmless endobiont of sea anemones (1). At the same time, oysters from both localities bore foliiculinid ciliates. These plankton feeders are characteristic of oyster shells from clean beds (69), for their tests are quickly blocked by bacterial jelly or accumulating ooze and sediments (2). The nature of their epibionts, and the constant presence of well-formed crystalline styles with abundant *Cristispira*, suggest that both batches of oysters originated in unpolluted waters. They did in fact do so. The Conway specimens had spent a short period in clean tanks associated with *Pecten maximus*, the shells of which likewise harbored few protozoa (*Lacrymaria coronata*, *Urostyla* sp., and unidentified colorless flagellates). They had been placed there following their removal from the Tal-y-foel oysterage

on the Menai Straits, which is at once protected from rough weather by a sandbank and subjected to daily tides (146) which keep pollutants from accumulating. The source of the Pakistan oysters was a harbor-mouth groin at Keamari, Karachi, where tidal scouring is again vigorous and regular.

No sickly oysters were seen at Conway or Karachi, and none of the healthy specimens examined (20 at each locality) bore any epibionts on the surfaces of the body itself. Unlike these individuals, oysters from New Brunswick and Prince Edward Island always had the shell liberally coated with black mud. This excluded *Folliculina*, but provided a substratum for numerous mesosaprobic to polysaprobic organisms (Table I), most of which favor low oxygen levels and tolerate the lowering of pH values accompanying an increase in hydrogen sulphide (60). Studies at Sam Orr Pond proved these organisms representative of the microfauna and microflora of the surface zone of the bottom mud on which, or just above which, rested the oyster trays. Certain inhabitants of the biological film also spread to the gills and other surfaces of the oysters themselves in both Canadian localities (Tables II and III).

Fifty seemingly healthy European oysters were examined at St. Andrews between 1958 and 1960. Thirty originated from Sam Orr Pond, 10 from the 1959 planting at Oak Bay, and 10 from a tray kept in the intertidal zone at the Biological Station wharf. Sickness was never apparent at the second and third sites (28, 95). All examples harbored one or more of four normally free-living protozoons on the gills and mantle. Two of these (*Amoeba radiosa*, *Monas guttula*) are not polysaprobic, one (*Rhynchomonas nasuta*) tolerates all levels of pollution, and the last, *Enchelyodon* sp., was identified from sick and decomposing oysters too. In each case the crystalline style was intact, with abundant *Cristispira balbianii*. The gut flora also included rare examples of *Spirillum* sp., close to if not identical with *S. virginianum* Dimitroff (11, 24), originally isolated from mud on the shell of a marketable American oyster at Baltimore. Polysaprobic preferences are usual in the genus (74), and the same *Spirillum* became abundant in sick European oysters and flourished in dead ones. Other bacteria present appeared to be those of the immediate environment (38).

The only protozoons found alive in the gut of healthy *O. edulis* were *Enchelyodon* sp., already reported from the same host in Europe (16, 17), and two dinoflagellates, *Prorocentrum micans* and the fresh- to brackish water *Glenodinium foliaceum*. The former is widespread in European seas and brackish waters in the spring and summer (19). It occurs abundantly on oyster grounds (131) and in the water of *O. edulis* (16, 17). At times it forms "red water" blooms (119), which can cause complete abortion of embryos and immature larvae by gravid oysters (78, 80). Nevertheless, at optimum concentrations, *P. micans* is undoubtedly an important food organism for oysters (93) and other lamellibranchs (36). Its summer occurrence under brackish conditions in Sam Orr Pond parallels that reported under similar circumstances on the Atlantic coast of the United States (23).

The contents of the digestive gland of healthy European oysters included other identifiable oligosaprobic plankters. Unlike the two dinoflagellates already mentioned, though, these were always dead and sometimes fragmentary. Armored dinoflagellates (*Dinophysis* spp.) less tolerant of brackish con-

ditions than the two species just discussed, and algae of the genus *Chlorella*, were prominent among them. So were  $\beta$ -mesosaprobic diatoms, such as *Nitzschia* spp., which form part of the normal diet of oysters and other filter-feeding marine invertebrates (43, 81), as do various other algae (18, 80) and bacteria of the bottom film (150). Ingested fungal spores and pollen grains, derived from the woods bordering the inlet, were sometimes common too.

As mentioned earlier, seven of these European oysters had *Ancistrocoma pelseneeri* on their gills, although in smaller numbers than did associated soft-shelled clams. The gills and palps of the latter occasionally exhibited scattered trichodonid ciliates as well, and *Urostyla* sp. was recorded once. The dominant molluscs of the Sam Orr Pond basin are quahags (*Mercenaria mercenaria*), common periwinkles (*Littorina littorea*), *Macoma balthica*, and the eastern mud snail (*Nassarius obsoletus*) (28). None of those examined bore any microorganisms on the body surfaces. In the gut of quahags and periwinkles, diatoms predominated over dinoflagellates (species of *Dinophysis*, *Glenodinium*, *Peridinium*, and *Prorocentrum*). Diatoms were likewise abundant among teeming bacteria in the gut contents of *Nassarius obsoletus*, a predator and scavenger.

European oysters from Sam Orr Pond differed from the indigenous molluscs with which they were associated in exhibiting the mortalities referred to in the Introduction. Twenty-seven species of microorganisms were present upon the body surfaces of the 40 gapers examined, more than five times the total for healthy oysters. All but three of the ciliates and *C. balbianii* were also found in the biological film over the substratum and the mud on the *O. edulis* shells (Table I). They included some two-thirds of the species collected from these sites. Of those not present on or within the oysters themselves, none are polysaprobic, and some (e.g., *Amoeba radiosa* and *Anisonema acinus*) probably prefer the rather higher pH values of a biological film in direct contact with alkaline brackish waters.

Nineteen of the species occurring on the gills and mantle of gapers were also found in the digestive gland, and three of them in the tissues. Gapers lacked a crystalline style, and any rare *Cristispira* were widely dispersed throughout the gut contents and over the external surfaces (113). The spirochaetes were in fact most plentiful on the body surfaces when present at all.

Fifteen normally free-living microorganisms common on the gills and mantle of gapers and identified to the species level (Table II) included one oligosaprobe to  $\alpha$ -mesosaprobe (*Holosticha kessleri*) and two  $\beta$ - to  $\alpha$ -mesosaprobic (*Aspidisca costata* and *Lionotus fasciola*). The remaining 12 comprised two-thirds of all the mesosaprobic to polysaprobic species encountered. All 11 microorganisms common in the gut or tissues of gaping European oysters (Table II) tolerate or prefer polysaprobic conditions.

Twenty dead European oysters were handled, ranging from examples in which portions of the ciliated epithelium were still feebly flickering to others that were mere blobs of putrefying matter. The number of microorganisms recorded from their surfaces had decreased from the 27 for gapers to 17, the only addition to the fauna being a saprobic rotifer of the genus *Diplax*. Eleven species were identified, all of them  $\alpha$ -mesosaprobic to polysaprobic. Finally, 13 of the microorganisms present on the exterior of these decomposing oysters were found in the interior too, eight of them commonly.

Downer (28) reported a cumulative mortality of 75% for a batch of the 1958 stock kept in a tank at the Biological Station. These European oysters exhibited heavy *Hexamita* infection, as did a giant scallop, *Placopecten magellanicus*, associated with them. The flagellate was also found in an American oyster that died in this tank, the bottom mud of which yielded scattered trophozoites of *H. inflata* towards the end of summer.

Turning now to Eilerslie and American oysters, 10 apparently healthy individuals, 10 gapers, and 10 dead ones were examined (Table III). Lack of time precluded paying attention to any associated molluscs or to the mud near the culture trays. The apparently healthy group bore no microorganisms on the gills or mantle, and the only ones found in the gut were rare examples of *Spirillum* sp. and abundant *Cristispira balbianii* in the crystalline style.

The body surfaces of gapers, though, harbored 21 microorganisms, some three-quarters of them recorded from mud on the shell (Table III). As in European oysters the crystalline style disappeared early, its *Cristispira* dispersing through the gut and becoming common on the gills and mantle. Besides the two parasites mentioned earlier (*Orchitophrya stellarum* and *Bucephalus cuculus*) the endobionts included various combinations of 14 of the 20 normally free-living species present on the body surfaces.

Thirteen of the species identified were common on the gills and mantle of gaping American oysters (Table III). They included one oligosaprobe to  $\beta$ -mesosaprobe (*Uronychia transfuga*) and four  $\beta$ - to  $\alpha$ -mesosaprobies (*Aspidisca costata*, *Bodo edax*, *Platynematum sociale*, and *Urostyla gracilis pallida*). The remaining seven, and the four microorganisms not specifically identified, all figure in the mesosaprobic to polysaprobic group recorded from gaping European oysters at St. Andrews.

The number of different microorganisms found on the surfaces of dead *C. virginica* had decreased from the 21 for gapers to 16, the only addition to the fauna being the polysaprobic *Dallingeria drysdali*. As was the case for European oysters, 11 species were identified. Apart from the occurrence of scattered examples of *Uronychia transfuga*, and the absence of *Sphaerotilus dichotomus* and *Acineta tuberosa foetida*, the list agrees with the equivalent one for *O. edulis*.

Twelve of the bacteria and protozoa present on the exterior of dead American oysters occurred in the gut or tissues too. Ten of them were classified as common, *Pleuronema marinum* and the nine so listed from the interior of dead European oysters too: *Spirillum* sp.; three flagellates, *Bodo rostratus*, *Dallingeria drysdali*, and *Hexamita inflata*; and five ciliates, *Cohnilembus verminus*, *Colpidium campylum*, *Cyclidium glaucoma*, *Euplotes charon*, and an unidentified tetrahymenid.

#### *Microorganisms Invading Living Tissues*

Living tissues were invaded by only three protozoans, *Hexamita inflata*, *Urostyla gracilis pallida*, and *Holosticha kessleri*. The first two were recorded from American oysters, and all three in European ones.

Neither of the ciliates is primarily a bacteria feeder or a carnivore. *H. kessleri* ingests diatoms and detritus as well as bacteria (148), and members of the genus *Urostyla* are all but omnivorous (130). However, on several occasions

both were observed to have followed *Hexamita* in invading the adductor muscle of the host. Their cytoplasm soon became crammed with ingested myolytic spindles, which, as previously described from sickly oysters (89, 113), were plentiful in the muscle tissue of gapers of both species.

#### *Hexamita inflata*

The infrequency with which *H. inflata* could be found in the biological film on mud near the oysters' trays and on their shells is in accord with the scarcity of field records for the flagellate. Nevertheless, Certes (16) found it not only in European oysters but in the surrounding brackish water too, and an earlier report from the Atlantic coast of North America (71) identified *Hexamita* sp. from bottom samples, debris, and centrifuged sea water. Although *H. inflata* has wide tolerances, population explosions are obviously restricted to rather a narrow range of conditions. A strongly reducing environment is not the only prerequisite for its appearance in large numbers. Though Liebmman (74) asserted that it occurs regularly and abundantly in almost every putrefying fluid, several of those who have studied sewage effluents and aging infusions have failed to find it. Perhaps, while occurring only in small numbers in alkaline (pH 7.2 to 8.4) brackish waters, it suddenly flourishes under polysaprobic conditions as the pH falls below a critical point in the acid range? During the present studies, its trophozoites certainly teemed, overshadowing associated microorganisms, at pH levels near 5.0.

All members of the genus *Hexamita* Dujardin being extremely small, and their most obvious features being the generic ones—the occurrence of two anterior nuclei, two axostyles, and six anterior and two posterior flagella—size and shape have been the chief criteria employed in differentiating the species from one another. Dujardin (30), who failed to distinguish the full complement of flagella, described *H. nodulosa* as the genotype. Bütschli (13) was probably correct in regarding this as merely a form of the second species established by Dujardin, *H. inflata*. Both exhibit numerous small vacuoles and refractive globules, the former being from 12 to 16  $\mu$  long, having small nodules which render its contours irregular, and appearing bifid posteriorly due to the cytoplasm being drawn out into tails along the trailing flagella. *H. inflata* was described as broadly oval, 17 to 20  $\mu$  in length, and posteriorly truncate, the hind margin being slightly concave between the points of exit of the widely spaced trailing flagella. The last-mentioned feature is highly characteristic and is clearly illustrated by Mackin *et al.* (89), whose identification was to the generic level only.

One hundred living trophozoites (from European oysters from Sam Orr Pond) were 11.5 to 18.9  $\mu$  in length and 6.8 to 11.7  $\mu$  in width (av., 14.4 by 8.5  $\mu$ ). Their trailing flagella measured from 18.9 to 33.4  $\mu$  (av. 26.1  $\mu$ ), being usually about twice as long as the body. By phase contrast many tiny vacuoles and glycogen globules were apparent, the axostyles showed clearly, and in a few especially favorable preparations viewed under oil a fluid appearance of the cytoplasm at the anterior extremity was suggestive of a cytostome. Fixed and stained examples showed shrinkage, measuring from 8.3 to 14.4  $\mu$  by from 4.9 to 10.3  $\mu$  (av., 11.7 by 6.6  $\mu$ ). The dimensions of living trophozoites overlapped the ranges of *H. nodulosa* and *H. inflata*, the smaller ones, recent

products of binary fission, often being of less regular outline than the others, the plump and posteriorly truncate appearance of which was in complete agreement with the latter species.

*H. inflata* was never common on the body surfaces of gapers, but its trophozoites occurred in huge numbers in the digestive gland, where Drinnan (29), too, observed them most often. *Trepomonas agilis*, another facultative anaerobe, sometimes accompanied it, these two flagellates frequently associating according to Kent (65). Infection soon spread to the gonad and blood vessels. As Orton (113) indicated when accounting for the presence of small flagellates (*Bodo* sp.) in the pericardium of gapers, this organ communicates with the exterior by way of the renopericardial canal and urogenital aperture, and relaxation of these passages in sickly oysters would permit microorganisms to pass from the mantle into the pericardium. The common invasion of blood by *Hexamita intestinalis*, a gut parasite of amphibians, is further evidence of the ease with which these flagellates penetrate one organ system from another.

As usual (89), *Hexamita*-containing leucocytes became concentrated in the blood channels, but there was no evidence of multiplicative stages within these cells corresponding to Davis' (21) intracellular schizonts of *H. salmonis*. Like Certes (16, 17), I observed only one method of multiplication, binary fission. There were no grounds for postulating that the presence of more than one rounded-up *Hexamita* in an individual leucocyte was due to anything other than phagocytosis. The ingestion of relatively large, active, and resistant trophozoites might well inhibit the normal migration of phagocytes from efferent blood vessels through surrounding tissues and the epithelial layers to the exterior (89, 138).

As death approaches, there is widespread necrosis of tissues surrounding the blood vessels, digestive gland, and intestine (89). This, the formation of trophozoite-packed abscesses, and the early invasion of the adductor muscle, all suggest the possibility that these flagellates secrete proteolytic enzymes. Having spread through the body, they continue to undergo binary fission and retain their normal activity for some time after the host's death. Indeed, they not only still teemed, but dividing individuals could still be found, in the putrefying remains of oysters dead for 24 hours. Such behavior, while to be expected of *H. inflata*, is hardly in accord with that of a specific parasite; neither are the facts that a giant scallop in a *Hexamita*-infested laboratory tank became as heavily involved as the oysters with which it was associated, and that the flagellate has also been recorded from quahaugs—not only from gapers, but from the liquid remains of a long-dead individual too (p. 474).

Numerous species of *Hexamita* have been described as internal parasites of insects, myriapods, fish, amphibians, reptiles, birds, and mammals. One has even been recorded from a trematode itself endoparasitic in eels (57). Nevertheless, specific parasitism must not be too hastily assumed where the host is aquatic and living under congested and otherwise abnormal conditions. Even if *Hexamita salmonis* (21) is an obligate parasite of trout transmitted, as claimed by Moore (97), via the egg, it does not follow that all *Hexamita* associated with hatchery epizootics or thriving in dead trout (144) are necessarily referable to the same species. Significantly, Davis (21) concluded that the factors most conducive to the development of *H. salmonis* are overcrowding

and underfeeding of the host, an unsuitable water supply, and oxygen deficiency. Pending morphological evidence to the contrary, the possibility of facultative parasitism by *H. inflata* should always be entertained, whether the aquatic host in question harbors its flagellates internally (144) or externally (109).

#### *The Protozoan Community of Putrefying Oysters*

The time of death of oysters defies normal definition, for microscopic examination may disclose active ciliated cells in individuals in advanced states of decomposition. For present purposes, though, a persisting gaper no longer manifesting co-ordinated life processes and clearly beyond any hope of resuming them is considered dead. At this stage several acidophilic protozoons, previously restricted to the body surface and gut contents, spread to the tissues. Notable among them are tetrahymenid ciliates, packed with refringent food spherules and conforming to the description of *Glaucoma scintillans* Ehrenberg. Recent work has shown the undesirability of positively identifying tetrahymenids without silver impregnation, which was not employed in this investigation. The determination is accordingly left open, although these ciliates cannot be differentiated from those found in polysaprobic liquids by several workers and assigned by them to *G. scintillans*. The species, which Orton (113) reported from the gut of dying oysters, has been identified from waters of a hydrogen ion content as high as pH 7.6 (7), but is best known from acid media. Kidder (66) gave its pH range as 5.6 to 6.8 and Šrámek-Hušek (135) recorded it at pH 6.1.

The other ciliates still prominent in both hosts as putrefaction proceeded were *Cohnilembus verminus*, *Colpidium campylum* (which, according to Kidder, grows best at pH 5.4), *Cyclidium glaucoma*, and *Euplotes charon*. This is evidently a highly characteristic community in decomposing organic matter in brackish water, for all its members were also common in secondarily infected external lesions on fish from such water at Singapore (original data, unpublished).

The bacteriophagous *Colpidium campylum*, *Euplotes charon*, and tetrahymenids remained present for 48 hours. By then *H. inflata* could no longer be found, the only flagellate left being a species characteristic of animal macerations, *Dallingeria drysdali* (65). Using nitrazine paper a pH value of approximately 5.0 was obtained from the putrefying remains. Hunter and Linden (59) gave figures of pH 4.6 to 5.0 for similar material, the bacterial flora of which they described in detail.

#### *Synopsis of Microorganisms Associated with Oysters*

To sum up, healthy oysters were found in the presence of oligosaprobic plankters and a variety of microorganisms of wide pollution tolerances. While their metabolism remained normal, they were unaffected by the proximity of saprobic bacteria and protozoa inhabiting the biological film covering their shells and the substratum. Relaxation of the adductor muscle, resulting from environmental deterioration, led to the appearance upon and within their bodies of many of the more acidophilic mesosaprobic and polysaprobic from the surrounding film. The community of microorganisms that now developed was characterized by well-known indicators of organic pollution. These

included "sewage fungus" (*Sphaerotilus dichotomus*), bacteriophagous and saprozoic colorless flagellates, and bacteriophagous (*Aspidisca costata*, *Chilodonea cucullulus*, *Cohnilembus verminus*, *Colpidium* spp., *Cyclidium glaucoma*, *Euplotes charon*, *Uronema marinum*, and tetrahymenids) and carnivorous (*Enchelyodon* sp., *Lionotus fasciola*, and *Uronychia transfuga*) ciliates—a community much resembling Picken's (118) "fungus association".

The rapid evolution of this complex community paralleled that of the bacterial flora forming the basis of the food chain. Those of its members with the least oxygen demand and highest acid tolerances were able to spread into the alimentary tract, with the continued fall in the resistance of oysters becoming progressively weaker under continuing anaerobic conditions. At some as yet undefined critical point, lowered host resistance led to the invasion of the blood and tissues by *Hexamita inflata*. With the exception of two ciliates (*Holosticha kessleri* and *Urostyla gracilis pallida*) that were sometimes associated with the flagellate in the adductor muscle, *H. inflata* was the only protozoon to enter the tissues before death took place. Afterwards, the liquefying remains of the host nourished a highly polysaprobic community comprising myriads of bacteria and large numbers (but relatively few species) of protozoa.

Unexplained mechanisms in the succession include the means whereby *H. inflata* overcomes host resistance and penetrates living tissues, and the relationships between it and ciliates joining in the invasion of living and dead tissues. The latter protozoa soon give place to bacteria after the disappearance of *H. inflata*, suggesting the possibility that this flagellate, like *Oikomonas termo* (51), renders conditions tolerable for certain ciliates by detoxifying bacterial metabolic products.

#### *An Ecological Background to Oyster Mortalities*

Such a cycle need not necessarily run its full course. It may be arrested or reversed if environmental conditions improve before the molluscs have lost the ability to resume normal metabolic activity, although whether they can, in fact, recover once the blood has been invaded by *H. inflata* is not known. Nor must its effects be uniform over wide areas or even over individual oyster bottoms. While the accumulation of excretory products and the excessive growth of microorganisms during periods of stagnation inevitably affect oysters adversely (10), mortalities are likely to be most pronounced where accumulations of organic pollutants are heaviest. Environmental pressure of this kind is part of the usual hazard of existence, occasioning continual scattered deaths just as do parasites and predators in balance with host populations. After all, cumulative mortalities of 10 to 20% are regarded as normal by oystermen (76, 113). Alarm is only occasioned by sudden rises above such levels, as at Sam Orr Pond between July 16 and 24, 1959. The cumulative mortality for 2-year-old and 3-year-old European oysters then jumped from 9% and 2.7% to 44.6% and 11% respectively, and all gapers teemed with *Hexamita*, which was much less evident both before and afterwards (28).

It is submitted that events like this are initiated by interruptions of the normal feeding activities of oysters, brought about by the high oxygen demand of pollutants, associated with a sudden rise in the level of bacterial activity in

the immediate vicinity. The numbers of saprobic organisms of all kinds rapidly increase, the rate of pumping decreases correspondingly (80), and shell closure is forced as a strongly reducing anaerobic environment develops (83).

At Sam Orr Pond, the initiating stimuli are linked with the facts that an entrance bar prevents tidal flushing during the neap phase, and that winter freezing takes place. During the neap phase, the oxygen level quickly falls to zero in the bottom zone of the basin. Thus on August 4, 1959, after an estimated 10 days without any flushing, no oxygen could be detected there, and on the 31st of that month, after only 6 days without flushing, the level had already fallen to 2.5 cc per liter (28). At these times, disturbance of the mud produced a strong smell of hydrogen sulphide. An ecological survey revealed patchy deposits rich in organic matter (28), the decomposition of which was accelerated by summer temperatures. A frequently overlooked factor contributing towards reducing activity in such deposits is that of self-silting by bottom-dwelling animals. Faeces and pseudofaeces, if not removed by external forces, accumulate rapidly enough to bury a single layer of oysters within 36 days (82, 83), and the annual wet weight of self-silt discharged from a single Japanese oyster raft has been estimated at 6 to 10 tons (60). A high percentage of this material being organic in origin, large amounts of free energy become available to microorganisms, and local bacterial decomposition is greatly accelerated.

There is a rapid decrease in the salinity of the surface layer of Sam Orr Pond during periods of stagnation. On August 24, 1959, immediately after flushing, the surface salinity was  $29.3\text{‰}$  (84% sea water), by comparison with a bottom reading of  $31.3\text{‰}$  (89% sea water). Two days later it was  $28.3\text{‰}$ , on the 28th  $17.5\text{‰}$ , and on the 31st only  $15.1\text{‰}$  (43% sea water). On the last-mentioned date, however, the bottom salinity stood close to the earlier figure, at  $31.2\text{‰}$  (28). Flushing following such a sequence has rapidly fatal consequences for purely marine plankters. From time to time macroplankters such as hydrozoan medusae and other coelenterates become very numerous in coastal waters, and jellyfish, among the more obvious marine invertebrates entering Sam Orr Pond on spring tides, quickly perish in the surface layer of too brackish water. Mortalities of this nature are less spectacular than the rotting of piles of stranded seaweed, which have been said to release gases directly toxic to oysters (126), but their significance is just as great. They cancel the temporary advantages—raising of the oxygen level and replenishment of the supply of oligosaprobic food organisms—derived by the oysters from tidal flushing of the inlet. With the onset of intense bacterial activity in the decomposing remains of macroplankters, the local environment again becomes a strongly reducing one. Once more the oysters must close their shells frequently, feed only sporadically, and cleanse themselves irregularly.

Any food intake in anaerobic periods must be rich in bacteria, and each interruption of feeding leaves great numbers of these not only throughout the gut but also over the body surfaces, particularly about the strings of mucus which normally transport food over the gills to the mouth (87). Oysters debilitated by pollution have the gills clogged by this mucus (107). As weakening of the adductor muscle from undernourishment and allied causes prevents proper closure of the shell valves, and gaping heightens exposure to predators

and infection (134), the bacteria-rich mucus mat over the gills invites invasion by bacteriophagous protozoa. Many of the microorganisms of the surrounding biological film thus move into this niche, some of them spreading to the digestive tract and tissues as already outlined. The ability of oysters to survive direct effects of an anaerobic environment (113) must inevitably break down should such conditions be too long maintained.

There is circumstantial evidence that they had been too long maintained at Sam Orr Pond just prior to the heavy mortalities of July, 1959. Downer (28) estimated that after June 27, 18 days went by without any flushing, the stagnant period culminating in prolonged anaerobism and overly high water temperatures. On July 16, Downer found that many oysters, although not yet gaping, felt "springy" when squeezed, indicating that the adductor muscle could no longer close the shell valves completely. That the subsequent death rate of 2-year-olds was four times that of 3-year-olds probably reflects earlier relaxation of the less developed adductor muscle in the younger oysters, leading to their longer exposure to the hazard of hexamitiasis—for the more efficient the valve closure of lamellibranchs during a period of acute danger of invasion by saprobic organisms, the better their prospects of surviving an anaerobic spell. Significantly, quahaugs were not involved in the outbreak. *Mercenaria mercenaria*, the most plentiful lamellibranch associated with the infected oysters, avoids undue bacterial invasion through being able to close its shell extremely tightly (27), and it will be remembered that even under the congested conditions of a laboratory tank where *H. inflata* occurred alike in European and American oysters, a giant scallop, and the bottom mud, the single quahaug present remained uninfected (28). Nevertheless, although able to resist infestation with microorganisms longer than oysters can, quahaugs are no less vulnerable to *Hexamita* once they are forced to gape. Mr. R. E. Drinnan (personal communication) has recorded the flagellate from three of them from Neguac, New Brunswick, two being lightly infected gapers while the third was reduced to a putrid liquid teeming with *Hexamita*.

With the onset of winter, bacterial decomposition proceeds in the self-silt that accumulates until water temperatures fall to a level (about 4° C) below which European oysters cease to cleanse themselves (25). The molluscs again become subject to the hazard of invasion by microorganisms, which takes place just as in summer if the adductor muscle begins to relax, as happens when sustained reductions of temperature to too low a level lead to loss of vitality (127). Low environmental temperature and not oxygen deficiency was held primarily responsible for the gaping of European oysters under the ice covering Sam Orr Pond in the winter of 1958–59 (28). Specimens then examined showed as heavy hexamitiasis as any seen in the summer, a very similar situation having been reported from Dutch holding basins, or pits (89). Once again, associated lamellibranchs remained free from facultative parasites. Quahaugs have already been discussed in this regard, and the other species examined, the soft-shell clam, is known to be able to cleanse itself of microorganisms at temperatures near freezing point (92).

The 100% mortality of European oysters at Eilerslie during the winter of 1958–59 was, as at Sam Orr Pond, associated with high incidence of *Hexamita* and temperatures of, and a little below, 0° C (29). Despite the severity of the

winter, equivalent mortalities did not take place among Prince Edward Island's native American oysters. Perhaps they escaped involvement through their capacity to continue normal metabolic activities at somewhat lower environmental temperatures than can *O. edulis* (39), and because of the superior efficiency of their cleaning mechanism (108).

Mackin *et al.* (89) reported that oxygen was 90 to 98% saturation and organic matter was normal during winter outbreaks of hexamitiasis among European oysters in Dutch holding basins. These facts are not at variance with the concept now presented. Even well-aerated waters support saprobic microorganisms, which are concentrated in a biological film so thin as not to affect the results of chemical assay of organic pollution. If oysters in stasis lose their resistance to invasion through failure to withstand prolonged low temperatures, mucus on their body surfaces is just as likely to attract bacteriophagous and other saprobes as it is in summer. Slower development of climactic polysaprobic communities will, of course, be evident than when the heavier organic pollution of warmer months enriches biological films as at Sam Orr Pond.

Mud thickly coated the American oysters examined at Ellerslie. The rich biological film on its surface proved to carry abundant microorganisms close to those found at Sam Orr Pond, and sickly and dead oysters harbored the same saprobes in both localities. Such evidence of environmental pollution was conspicuously absent when oysters from clean bottoms were inspected during similarly brief visits to Conway and Karachi. It is therefore suggested that future studies of "Malpeque disease" should be so conducted as to exclude any possibility of confusion between deaths due to an unknown pathogen and those simply hastened by facultative parasitism under environmental conditions unsuited to the maintenance of stable oyster populations.

#### *Environmental Pollution and Past Oyster Mortalities*

Declines in oyster populations have sometimes been attributed to sewage or industrial wastes, notably pulp-mill effluents. A great deal more notice has, however, been taken of the direct effects of these pollutants upon oysters than of secondary effects upon the microenvironment. Published observations nevertheless permit some speculation about the contribution of the latter to abnormally high death rates.

One of the clearest indications of the association of alarming oyster mortalities with deterioration of the immediate environment through gross pollution concerns the Taranto disaster of 1919-20. Orton (113) quotes Professor Cerruti for the information that these beds became contaminated by sewage effluents from World War I military camps and heavy shipping. The anaerobic conditions that developed were worsened by the release of free  $H_2S$  and a heavy silt suspension during a naval salvage operation. This combination of circumstances, itself sufficiently detrimental to oysters (60), terminated in heavy infestation of the latter with filiform bacteria. It is a legitimate assumption that polysaprobic protozoa characteristic of the *Sphaerotilus* association were abundant too.

Industrial wastes have been blamed for weakening the adductor muscle of oysters and causing gaping (107). Galtsoff *et al.* (42) and McMillan (88) both reported that the highest oyster mortalities due to pulp-mill effluents coincided

with the highest sulphate levels, and it is suggested that the biological film at the bottom of these zones of vigorous chemical oxygen demand was characterized by an abundance of *Beggiatoa alba*. This species, another of the sewage bacteria, is very prevalent in sulphate-rich brackish water. It coats mud surfaces with a whitish film the ciliates of which have been studied by Fauré-Fremiet (34). The growth is first exploited by the bacteriophagous *Colpidium campylum* and *C. colpoda*, which attract predaceous ciliates (*Enchelys*, *Lionotus*, *Leucophrys*), an association succeeded by *Chilodonella cucullulus* and tetrahymenids. While low concentrations of pulp-mill effluents stimulate the growth of *Nitzschia* (42) and *Melosira* (88),  $\beta$ -mesosaprobic diatoms in the presence of which oysters develop well, hexamitiasis is thus a likely event when high concentrations favor the development of polysaprobic communities. Schodduyn (131) associated summer losses in a European oyster park with the appearance of a *Beggiatoa* mat.

Fresh-water dilution, too, has had most attention from the standpoint of its direct effects upon oysters. Thus Galtsoff (40) attributed an epizootic in Mobile Bay, Alabama, to a highly unusual coincidence of heavy local rainfall with the flooding of rivers discharging into the Bay. Again, Orton (115) considered high mortalities of *O. edulis* in the British Isles due to the "continuous freshet that might have followed the heavy autumnal rains of 1934", and the death of up to 75% of American oysters in parts of Chesapeake Bay in 1945-46 was ascribed to the fact that salinities had fallen to only one-half to one-third of normal (61). U.S. Fish and Wildlife Service data show five peaks of oyster mortality in upper Chesapeake Bay during the previous 38 years. All were reached at times when heavy rains had caused the Susquehanna River to reach an abnormally high level, salinity in the Bay being much reduced in consequence (4).

As to indirect effects of unduly fresh conditions upon oysters, heavy Australian casualties have been ascribed to bacteria thriving in *Polydora* blisters following the death of the worms through fresh-water exposure (149), and Roughley (127) reported lesions resembling those due to hexamitiasis in oysters subjected to freshets in New South Wales.

Inshore salinity reductions also marked the severe British mortalities of 1920-21. None of the accounts of events of this sort consider the possible contribution to organic pollutants through the wholesale death of fully marine organisms brought in by tides. Yet such plankters are attuned to remarkably stable salinity conditions, and all typically open sea species perish when subjected to drastic reductions in the chloride level (20). So, for that matter, do European oysters, which, although their deaths may be hastened by invading saprobic microorganisms, are highly unlikely to survive extended exposure to an unduly freshened environment. The latter alone largely explains certain notable failures of oyster fisheries. For example, the rapid decline of the Firth of Forth beds in the late 17th century, when European coastal salinities fell due to the rains that coincided with the retreat of the Chamonix glaciers; and the dwindling production from English and Norwegian oyster bottoms during the abnormally wet 1870's (137). *Hexamita inflata* must have flourished at these times and during the British mortalities of 1920-21, but only in the rôle of a scavenger except towards the periphery of its range, where, although

organic decomposition was more than usually pronounced, depth or distance from the shore minimized salinity reductions. There, the flagellate might well have ensured the death of many oysters that could otherwise have survived.

The latter rôle of hexamitiasis is regarded as of most importance to European oysters grown or overwintered under marginal conditions. It is perhaps of even greater significance to American oysters, which are normally cultured in bays and estuaries. Following prolonged rains and flooding, organic material of both marine and fresh-water origin accumulates on estuarine bottoms. *Crassostrea virginica* tolerating the physical effects of a considerable measure of dilution (examples have survived exposure to fresh water for several weeks) is it not conceivable that failure to survive freshets is often due to the activities of pollution microorganisms including *H. inflata*? This suggestion is supported by information supplied to Orton by the then U.S. Commissioner of Fisheries, who wrote (113) that, during the Chesapeake Bay mortalities of 1916, the zone about the dying oysters was anaerobic and unusually rich in decomposing organic debris.

### Conclusions

Oysters, like other animals, play host to a variety of true parasites. When they exhibit abnormally heavy mortalities from unknown causes, it is therefore tempting to postulate parasitic scapegoats, the choice of which is influenced by prevailing fashions in research. In the latter nineteenth century a bacterium had to be responsible for "Maladie du pied"; when protozoology held the stage early in our own century it was inevitable that an undiscovered protozoon would be proposed as the possible cause of epizootics in Norwegian oyster ponds (49); and more recently, there have been suggestions that a virus may be to blame. The fact remains that no studies of major oyster mortalities have yet proved any specific parasite of first importance per se (134), and it has been contended that such parasites only assume grave significance when general environmental deterioration so lowers host resistance as to transform regular, low-level loss into disaster (145).

Reference to the extensive literature on the vicissitudes attending the culture of oysters suggests that too often, as in the extreme example of Sam Orr Pond, they are grown under conditions inviting intermittent disasters. After all, the oysters with which this paper is concerned are reef, not bottom, animals (103). Granted favorable temperatures and food availability, they grow best on a hard substratum, not a muddy one (28), in places where little silt can accumulate (22). Optimal sites for both species are just below low-water mark and subject to daily tidal influences, for constant submergence offers the steadiest growth of shore lamellibranchs (22, 146, 147) while regular scouring minimizes pollution hazards.

Such sites are admittedly apt to be exposed to higher levels of predation (147) than brackish waters. If the latter are relatively shallow their summer warmth will combine with an abundance of food material to ensure good growth of oysters physiologically suited to them, and at the same time they provide especially favorable harvesting conditions. American oysters in particular are thus commonly cultured in sheltered estuaries under conditions obviously advantageous to the growers, who, knowing that their unnaturally congested

stock can recover from the direct effects of temporary exposure to physiological adversities encountered there, have become aware of only part of the hazard of pollution in waters of this kind.

There are several roads to the polysaprobic zone, and neither high levels of *Escherichia coli* nor toxic industrial effluents, the two factors usually equated with the term "pollution", are necessarily prerequisite to the development of saprobic communities. Less obvious natural pollution through the accumulation of self-silt or of the remains of organisms killed by sudden salinity changes, is easily overlooked. Analysis of samples from the water mass overlying oyster bottoms can altogether fail to detect pollution of this order, leading to claims that the level of organic matter is tolerable when, in fact, biological assay of the film in the immediate vicinity of the molluscs themselves would show that it is not. Saprobic microorganisms are always present in aquatic habitats. Rotting oysters, like any other organisms dying there, will yield them in abundance even when a high level of cleanliness ensures their relative rarity in the biological film over the substratum (131). It is only when they begin to flourish in the immediate environment of living oysters, that communities of the Saprobiensystem assume significance as biological evidence of pollution, a fact that justifies repetition of a recent warning that those concerned should "make special efforts to examine in all possible detail the factors which interact to make a particular environment more or less suitable for oysters" (12).

It is fully appreciated that the original data presented herein are not only scanty but mainly qualitative. Nevertheless, considering them against the background of a considerable literature, it is believed that they support certain general conclusions:

(a) that when temperature, rainfall, or tidal factors render a local environment physiologically unfavorable for oysters, these become progressively less resistant to invasion by saprobic microorganisms;

(b) that hexamitiasis results from facultative parasitism by one of these microorganisms, *Hexamita inflata*;

(c) that locally anaerobic conditions predisposing to hexamitiasis are indicated by the flourishing of communities of the Saprobiensystem in the immediate vicinity of living oysters and within the shells of sickly individuals;

(d) that unless environmental conditions improve before such oysters lose the ability to recover, there will be abnormal mortalities, hastened, though not primarily caused by, the invading microorganisms;

(e) that micropollution and hexamitiasis are contraindicated by the abundance of *Cristispira balbianii* in a well-developed crystalline style, and of folliculinid epibionts.

As to the practical significance of these conclusions, it is obvious that nothing can be suggested for the protection of oysterages normally kept clean by tidal flushing and located in narrows or on exposed coasts where dilution can only become a problem through freshets of a magnitude reached perhaps two or three times in a century. Such oysterages, like those near Conway and Karachi, have long trouble-free histories. These are sites where the oyster species concerned thrive best in nature, and only the impact of rare and profound environmental crises is likely to cause major population fluctuations.

If oyster hexamitiasis is indeed simply an end point in a chain of events initiated by deterioration of the environment, there is clearly no general remedy for the condition. Each outbreak poses its own special problems, cycles in the community of the bottom biological film being influenced by the various stimuli described in the preceding pages and undoubtedly by others too, for example periodic tide-induced fluctuations in the concentrations of potassium and other elements, affecting the activity of marine organisms (41). Just as Davis (21) remarked regarding *H. salmonis*, the havoc caused by hexamitiasis is in inverse ratio to the suitability of the environment. Much could be done to render it less hazardous by improving culture techniques. It is timely to recall a remark made long ago by Dr. P. C. C. Hoek and quoted by Korringa (68), namely that oyster culture *is* a culture and not a manufacture.

The results of studies of the tolerances of oysters have tended to lead to the intensive exploitation of areas chosen by growers, rather than towards an understanding of the conditions optimal for these animals in nature. The concentrated research into these conditions urged by a few investigators in recent years (12, 145) has yet to be undertaken, and is again advocated.

Present data suggest that solutions for local problems would include the elimination of overcrowding on oyster beds, the free use of shell to harden the muddy bottom of tidewater ponds (86), and regular removal of self-silt and other deposits of high organic content where conditions favor their rapid accumulation. This could be effected by dredging, already employed with encouraging results in Holland (69), and resorted to in combination with scraping for the removal of mud crabs (the primary hosts of the gregarine, *Nematopsis ostrearum*) from some American beds (53). Rotation of oyster crops and bottom ploughing—of more limited application than suction dredging, being dependent on tidal exposure (60)—also offer possibilities in suitable situations. Improved techniques, particularly for the control of predators, might justify the development of new oysterages on the open coast where micropollution problems are minimal.

Intensive biological research, besides revealing how best to exploit oysters native to particular areas, is the only means of providing a sound basis for the establishment of chosen stocks in new localities. Perhaps other strains of European oysters than the Conway one might prove more adaptable to a polluted and muddy environment. After all, the species has adapted itself to widely differing conditions from Norway to the Aegean, and hybridization and selection could be exploited in this connection (48). Again, oysters differ among themselves as regards the efficiency of valve closure. By analogy with the relative freedom from microbial invasion exhibited by other lamellibranchs associated with European oysters at Sam Orr Pond, as yet unconsidered species of oysters might prove more adaptable to Canadian Atlantic coast conditions. The practicability of using air transportation in effecting new introductions having now been clearly demonstrated, no bar remains to experimenting with candidate species (Australasian mud oysters for example) from the most distant sources.

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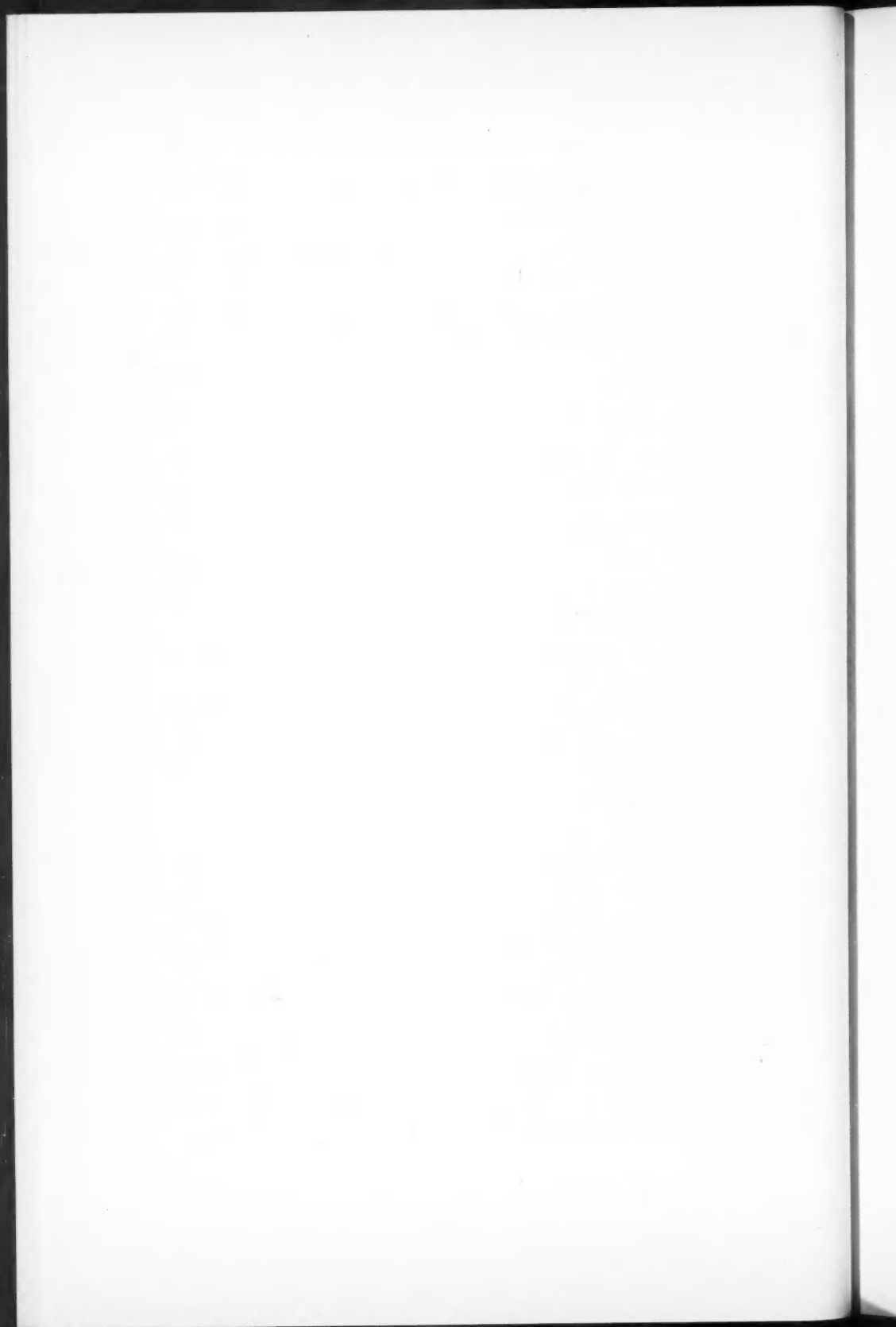
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## HYBRIDIZATION EXPERIMENTS IN RHODEINE FISHES (CYPRINIDAE, TELEOSTEI)

### THE INTERSPECIFIC HYBRIDS OF *RHODEUS OCELLATUS* FROM JAPAN AND KOREA, AND *RHODEUS SPINALIS* FROM TAIWAN<sup>1</sup>

J. J. DUYVENÉ DE WIT

#### Abstract

In an attempt to elucidate the phylogenetic relations between rhodeine species a number of interspecific and intergeneric crossings have been performed. This publication deals with the progenies obtained from the combinations *Rhodeus ocellatus* (Japanese strain)  $\times$  *Rhodeus spinalis*, *Rhodeus ocellatus* (Korean strain)  $\times$  *Rhodeus spinalis*, and *Rhodeus ocellatus* (Japanese strain)  $\times$  *Rhodeus ocellatus* (Korean strain).

With the exception of one  $F_1$  in which no males were produced, the progenies of the other five combinations consisted of fertile males and females, representing fertile interbreeding populations by producing fry in the presence of freshwater mussels.

In all combinations more females than males were present.

#### Introduction

With a view to investigating the presence and the degree of genetic affinity possibly existing between representative species of rhodeine fishes, a number of hybridization experiments have been performed under laboratory conditions.

In a recent paper the successful hybridization between *Rhodeus ocellatus* (Japan)  $\varnothing$   $\times$  *Acheilognathus lanceolata* (Japan)  $\sigma$  and the reciprocal combination has been reported by the present author (1). The two kinds of hybrids obtained from this combination showed a male phenotype. Although sperm production was very much impaired in these hybrids, some viable larvae have been obtained by inseminating eggs of both parental species with sperm of these fishes.

Intergeneric hybrids between *Acheilognathus lanceolata*  $\varnothing$   $\times$  *Acanthorhodeus atremius*  $\sigma$  and *Tanakia tanago*  $\varnothing$   $\times$  *Acheilognathus rhombea*  $\sigma$  have also been produced (Duyvené de Wit (2)). From the first combination adult hybrids which showed a male phenotype have been obtained, while in the second combination only larvae which died as a result of starvation, caused by a deficient supply of yolk, were produced.

Intergeneric hybrids between *Rhodeus ocellatus*  $\varnothing$   $\times$  *Rhodeus sericeus amarus*  $\sigma$  (from western Europe) and the reciprocal combination were also described by the present author (3). Both kinds of hybrids showed a male phenotype. In the spawning season they displayed full nuptial colors and spawning behavior, but milt production by means of stripping could not be observed.

The specimens of *Rhodeus spinalis* (Oshima) used in our present experiments were imported from their natural habitat, Taiwan. The specimens of

<sup>1</sup>Manuscript received March 28, 1961.

Contribution from the Department of Zoology, University of the Orange Free State, Union of South Africa.

*Rhodeus ocellatus* (Kner) belonged to two geographically isolated strains, one originating from Japan and the other from Korea.

When the phenotypes of the three present strains of bitterlings are compared morphologically, slight differences between both the Korean and Japanese strains of *R. ocellatus* are apparent, while the Taiwan species *R. spinalis* is fairly similar to both strains of *R. ocellatus*. We are inclined to regard the present three strains as geographical varieties belonging to one single species and we intend to discuss the subject in a future paper.

Because of the similarity between *R. spinalis*, *R. ocellatus* from Japan, and *R. ocellatus* from Korea, the question arose whether hybrid offspring could be obtained from these strains by means of natural interbreeding, and whether, in contradistinction to the first-mentioned crosses from which only male phenotypes were obtained, the different progenies would consist of fertile males and females as well.

### Materials and Methods

In Table I the six possible breeding combinations between individuals of the three strains are indicated with the numbers 1-6.

Each combination consisted of two males and four to six females. The fishes were placed in well-aerated aquaria provided with three to five fresh-water mussels (*Aspatharia wahlbergi* Krauss and *Unio caffer* Krauss). They were fed deep-frozen fish roe and a commercial dry compound preparation which meets the requirements of omnivorous fishes.

TABLE I

	<i>R. ocellatus</i> (Japan) ♂	<i>R. ocellatus</i> (Korea) ♂	<i>R. spinalis</i> ♂
<i>R. ocellatus</i> (Japan) ♀		1	3
<i>R. ocellatus</i> (Korea) ♀	2		5
<i>R. spinalis</i> (Taiwan) ♀	4	6	

### Results

In all six combinations spawning occurred readily and the first larvae appeared 5-6 weeks after the commencement of the experiment. Two months later the adult fishes were removed from the aquaria and the fry were allowed to develop until sexual maturity was reached. The following results were obtained.

#### 1. *R. ocellatus* (Japan) ♀ × *R. ocellatus* (Korea) ♂

This combination yielded 13 males and 29 females. By means of stripping, milt was abundantly produced by the males, and eggs were extruded by the females. A representative female and male specimen of the present  $F_1$  is illustrated in Fig. 3. A female specimen of *R. ocellatus* from the Japanese

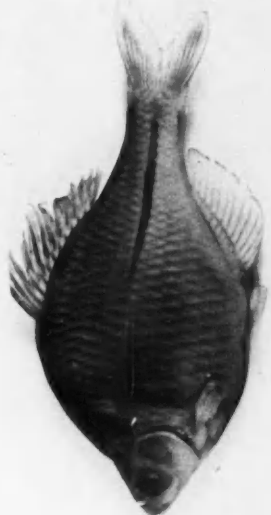
FIG. 1. Female specimen of *Rhodeus ocellatus*, Japanese strain. Standard length 44 mm.

FIG. 2. Male specimen of *Rhodeus ocellatus*, Korean strain. Standard length 61 mm.

FIG. 3. A female and a male specimen obtained from the combination female *R. ocellatus* (Japan) × male *R. ocellatus* (Korea). Standard lengths 44 mm and 52 mm respectively.

FIG. 4. Female specimen of *Rhodeus ocellatus*, Korean strain. Standard length 14 mm.

PLATE I



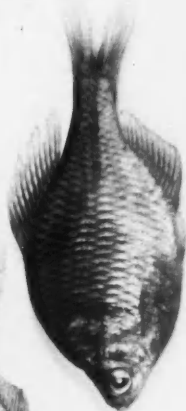
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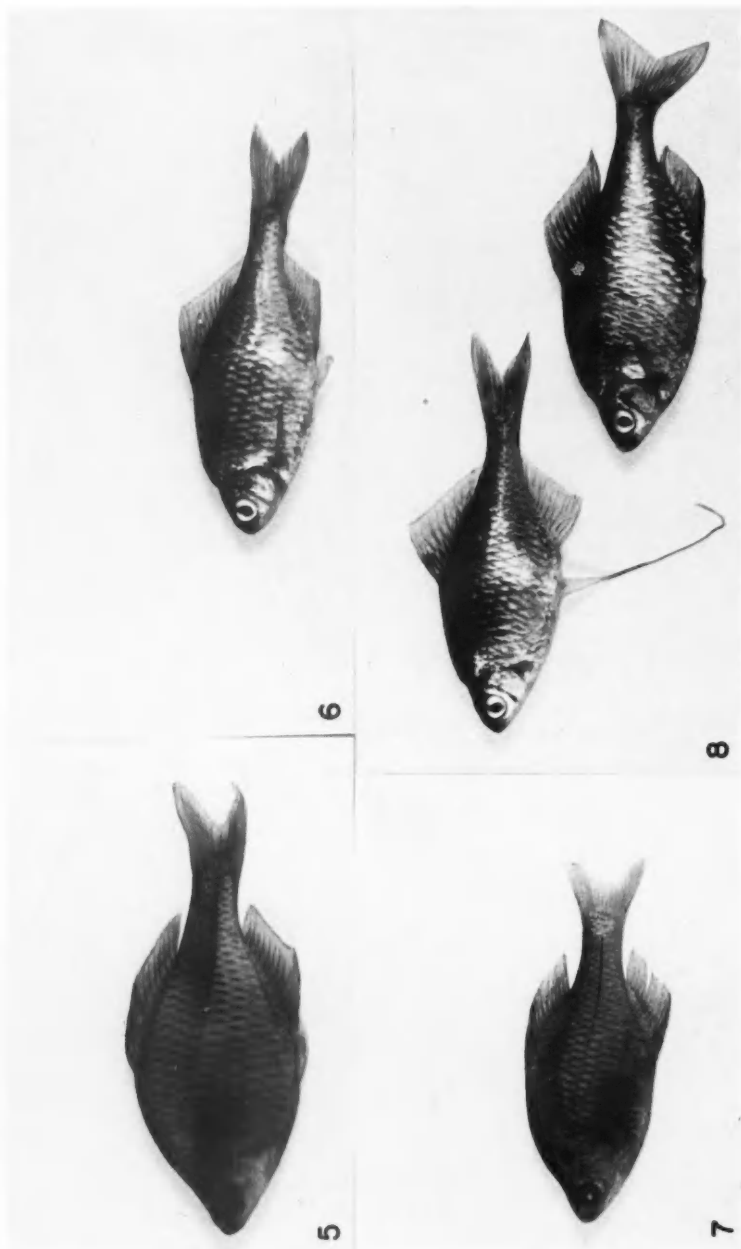
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3

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PLATE II



strain is illustrated in Fig. 1 and a male specimen of the Korean strain of this species in Fig. 2.

2. *R. ocellatus* (Korea) ♀ × *R. ocellatus* (Japan) ♂

From this combination, which is the reciprocal of the former one, seven females were obtained. The outbreak of an infectious disease during the rearing of the fry accounts for the relatively small number of progeny, while the absence of males is probably accidental. By means of stripping, eggs were extruded by all specimens. A representative female of the present  $F_1$  is illustrated in Fig. 6. A female specimen of the Korean strain of *R. ocellatus* is illustrated in Fig. 4 and a male specimen of the Japanese strain of the same species in Fig. 5.

3. *R. ocellatus* (Japan) ♀ × *R. spinalis* ♂

From this combination 8 males and 15 females were obtained. In all males milt production was abundant and from all females, eggs could be collected. In Fig. 8 a representative female and male specimen of the present hybrids is illustrated. A female specimen of the parental species *R. ocellatus* from Japan is illustrated in Fig. 1 and a male specimen of the parental species *R. spinalis* from Taiwan in Fig. 7.

4. *R. spinalis* ♀ × *R. ocellatus* (Japan) ♂

This combination is the reciprocal of the former one. Due to an infectious disease which killed the greater part of the fry, only one male and four females reached sexual maturity. Milt and egg production were as abundant as in sexually mature specimens of both parental species. The male specimen and one of the female specimens of the present hybrids are illustrated in Fig. 10. A female specimen of *R. spinalis* is illustrated in Fig. 9 and a male specimen of *R. ocellatus* of the Japanese strain in Fig. 5.

5. *R. ocellatus* (Korea) ♀ × *R. spinalis* ♂

From this combination 14 males and 20 females were obtained. Milt and egg production were as abundant as in both parental species. A representative female and male specimen of the present hybrids is illustrated in Fig. 11. A specimen of the maternal species *R. ocellatus* from Korea is illustrated in Fig. 4 and a specimen of the paternal species *R. spinalis* in Fig. 7.

6. *R. spinalis* ♀ × *R. ocellatus* (Korea) ♂

From this combination, which is the reciprocal of the former one, three males and six females could be reared to sexual maturity. One male and two female specimens remained weak as a result of an infectious disease which killed a number of hybrids when only half-grown. In the remaining specimens milt and egg production were as abundant as in both parental species. A representative female and male specimen of the present hybrids is illustrated in Fig. 12. A female specimen of *R. spinalis* is illustrated in Fig. 9 and a male specimen of *R. ocellatus* of the Korean strain in Fig. 2.

FIG. 5. Male specimen of *Rhodeus ocellatus*, Japanese strain. Standard length 57 mm.

FIG. 6. A female specimen obtained from the combination female *R. ocellatus* (Korea) × male *R. ocellatus* (Japan). Standard length 50 mm.

FIG. 7. Male specimen of *Rhodeus spinalis* from Taiwan. Standard length 46 mm.

FIG. 8. A female and a male specimen obtained from the combination female *R. ocellatus* (Japan) × male *R. spinalis*. Standard lengths 49 mm and 54 mm respectively.

### Discussion

In the introduction it was stated that genetic affinity, or at least gametic compatibility, does exist between the western European species of bitterling *Rhodeus sericeus amarus* and the Japanese species *Rhodeus ocellatus*. This phenomenon is striking considering the rigorous isolation in space and time to which both species have been subjected. It is true that the hybrids obtained from this combination consisted of male phenotypes only, whose gonadal activity was greatly impaired, thus indicating that the genomes of both parental species had lost complete compatibility.

From our present investigations it appears that the six kinds of offspring resulting from the six possible cross combinations between *R. spinalis* (Taiwan) and *R. ocellatus* (Korean and Japanese strains) consisted of both fertile males and fertile females. (It is presumed that the relative small number of surviving fishes in combination 2 accounts for the absence of males.)

Actually, in the presence of fresh-water mussels, the  $F_1$ 's of the combinations 1, 3, 4, 5, and 6 behaved as fertile experimental interbreeding populations and fry were abundantly produced. Lack of accommodation prevented us from rearing them to maturity in order to produce further generations.

Yet the unbalanced sex-ratio, which manifests itself in the six kinds of  $F_1$ 's obtained in our experiments, possibly suggests the presence of incompatibility between part of the gametes or genomes of the relevant parental species. The expected sex-ratio is 1:1, but in all  $F_1$ 's the number of females exceeds that of the males. The gross sex-ratio is about one male: two females, thus suggesting a 50% mortality in the male zygotes.

We intend to examine this question by artificially cultivating fertilized eggs of the present combinations in glass dishes and establishing the rate of non-developing eggs and embryonic mortality in these cultures.

A detailed taxonomic description of the six types of offspring under discussion here will be published separately.

### Acknowledgments

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PLATE III

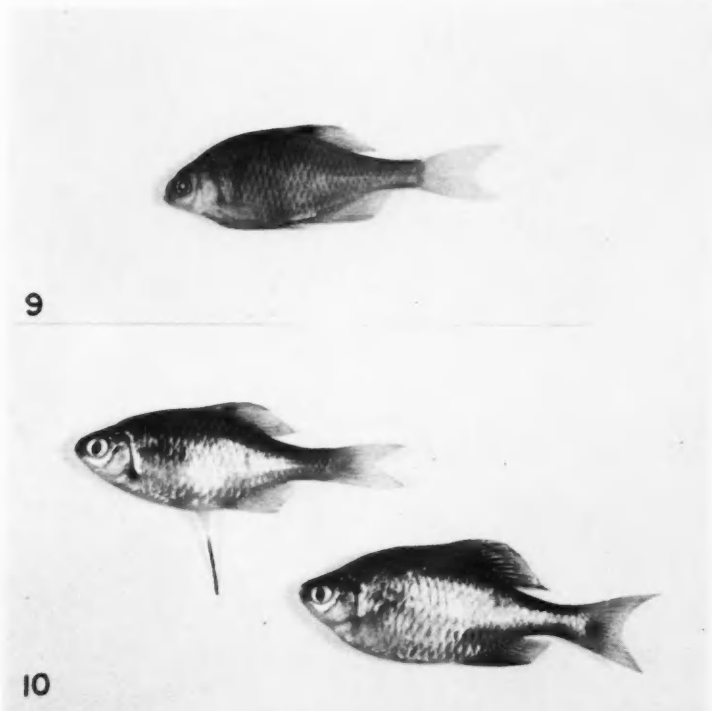


FIG. 9. Female specimen of *Rhodeus spinalis* from Taiwan. Standard length 39 mm.  
FIG. 10. A female and a male specimen obtained from the combination female *R. spinalis*  $\times$  male *R. ocellatus* (Japan). Standard lengths 40 mm and 44 mm respectively.

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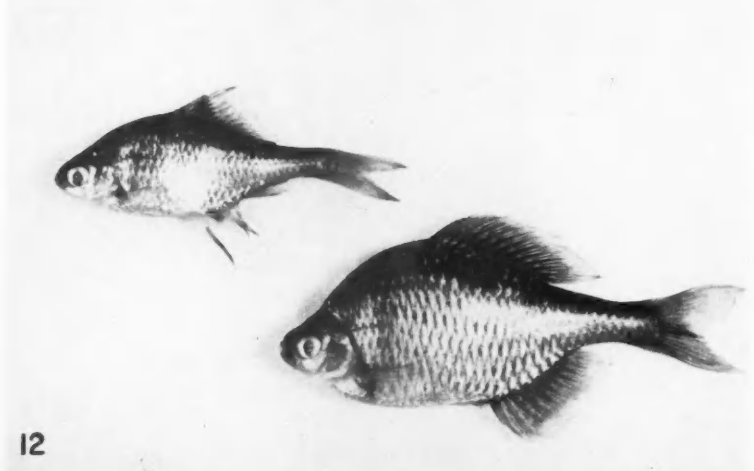
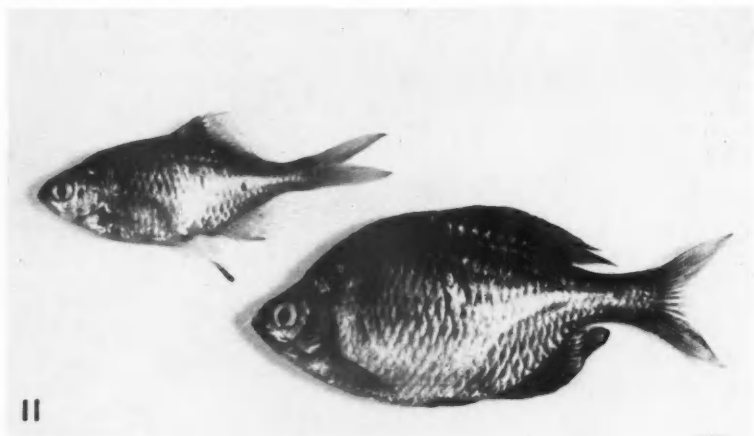


FIG. 11. A female and a male specimen obtained from the combination female *R. ocellatus* (Korea)  $\times$  male *R. spinalis*. Standard lengths 41 mm and 61 mm respectively.

FIG. 12. A female and a male specimen obtained from the combination female *R. spinalis*  $\times$  male *R. ocellatus* (Korea). Standard lengths 43 mm and 60 mm respectively.

Duyvené de Wit—Can. J. Zool.

## EMBRYOLOGICAL DEVELOPMENT OF A NONDIAPAUSE FORM OF *MELANOPLUS BILITURATUS* WALKER (ORTHOPTERA: ACRIDIDAE)<sup>1</sup>

P. WM. RIEGERT<sup>2</sup>

### Abstract

A pictorial and written description of the external morphology of embryos of a nondiapause form of *M. bilituratus* is given. Daily morphological changes, on each of the 17 days of embryological development, are recorded and related to the duration of incubation at 30° C. Development at 25° and 35° C was completed in 24 and 15 days, respectively.

### Introduction

In 1949 Salt (6) published a key to the embryological development of three species of grasshoppers, *Melanoplus bivittatus* (Say), *M. mexicanus mexicanus* (Sauss.),<sup>3</sup> and *M. packardii* Scudder. Other authors (1, 2, 3, 4, 5, 7, 9) had carried out similar investigations both before and after this date, but the species involved were either Old World forms or were not indigenous to the prairie region of Canada. The three species mentioned above, as well as many others including locusts, are univoltine species with an obligatory diapause occurring during the later stages of embryogenesis. Thus an embryological timetable or schedule of development was an interrupted one and was therefore made by inference and/or by extrapolation.

At the Saskatoon Research Station a nondiapause form of *M. bilituratus* was obtained through selection and rearing of nymphs that hatched from nondiapause field-collected eggs. (This type of egg is always present in very low numbers within any natural field population.) Rearing through 12 generations produced a fairly stable nondiapause population. The embryological development of this form was studied continuously and chronologically from the time of oviposition to hatching, thereby giving a schedule of development that was uninterrupted by diapause. The external changes of the embryos, as related to the duration of incubation at 30° C, are presented in this paper.

### Materials and Methods

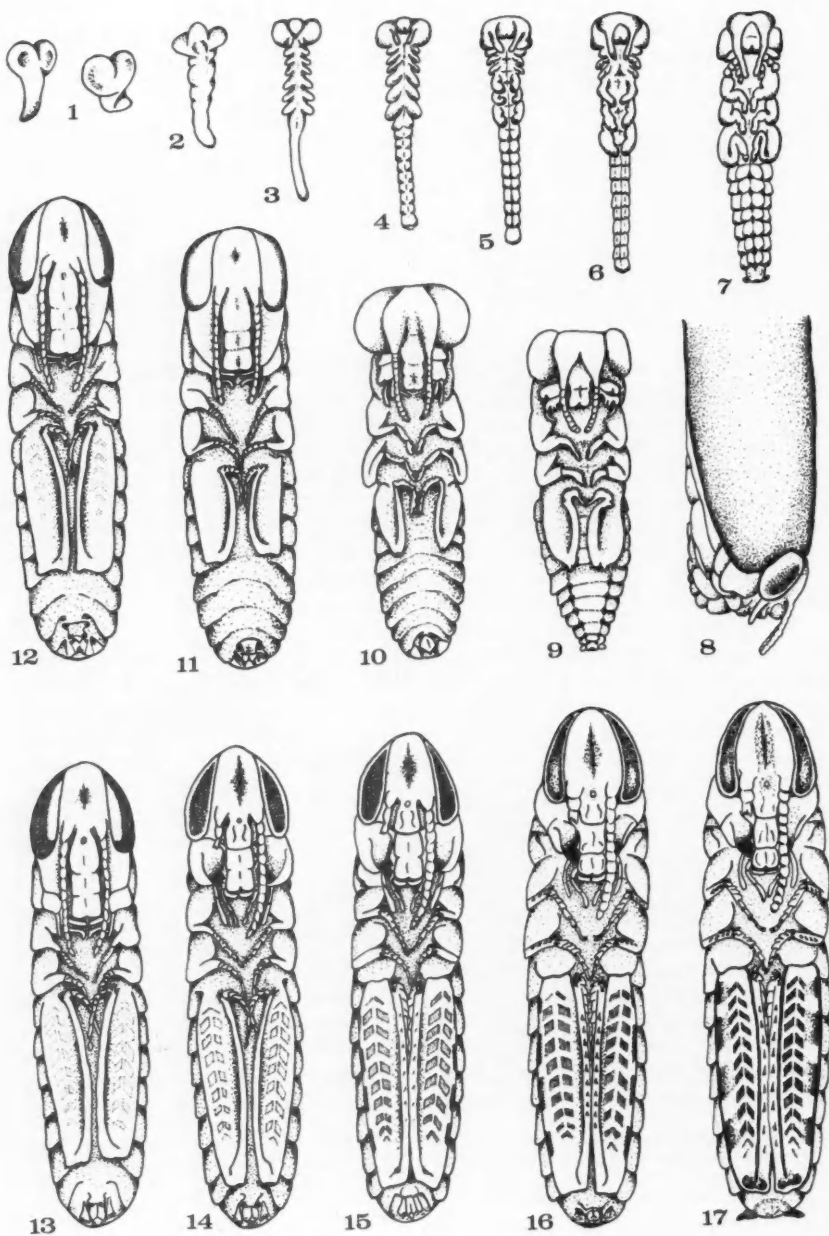
Grasshopper eggs, collected daily from laboratory cultures of adults, were placed in sterile quartz sand in small, sterile Petri dishes and incubated at 30° C. The eggs were kept moist with distilled water containing methyl *p*-hydroxybenzoate, a fungistatic agent, to make an approximately 0.01% solution. Samples of eggs, taken daily, were examined for embryological development after the chorion had been removed in a solution of sodium hypochlorite (8). The embryos were quite clearly visible, *in situ*, under a binocular microscope when transmitted light was passed obliquely through

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<sup>3</sup>Presently known as *M. bilituratus*.



the egg. Drawings of the embryos were made using a squared-field ocular. A description of the daily morphological changes of embryonic growth is given below.

### Results

Figures 1 to 17 show the average daily stages of growth of the embryos at 30° C. The variations from these averages were slight, indicating a rigid schedule of development. Approximately eighty per cent of about two thousand eggs hatched on the 17th day of incubation; about twenty per cent, on the 18th; a few eggs from three different batches, on the 16th; and about half of the eggs in one pod hatched on the 15th day.

#### *Schedule of Development of Embryos*

- 1-Day.—*Blob of cells*,<sup>4</sup> sometimes with a short 'tail'; Fig. 1.
- 2-Day.—Head lobed; 'tail' *about three times as long as head*, partly segmented; Fig. 2.
- 3-Day.—Optic lobes distinct; *rudiments of head appendages distinct*; legs distinct; no segmentation of abdomen; Fig. 3.
- 4-Day.—Antennae elongate; labrum partly fused; *legs bulbous*; abdominal segmentation incomplete; Fig. 4.
- 5-Day.—*Legs turn inward*; superficially jointed, more bulbous than tubular; segmentation of abdomen complete; Fig. 5.
- 6-Day.—Head appendages jointed; legs large, bulbous, jointed; *tergo-sternal suture of abdomen indistinct*; Fig. 6.
- 7-Day.—*Hind legs N-shaped*; labial palps well formed; antennae segmented; tergo-sternal suture prominent; anatrepsis completed; Fig. 7.
- 8-Day.—*Revolution of the embryo*; eye crescents appear faintly; Fig. 8.
- 9-Day.—*Beginning of katatrepsis*; *short, squat embryo* less than half the length of egg; Fig. 9.
- 10-Day.—*Embryo half the length of egg*; femora spindly, shorter than antennae; Fig. 10.
- 11-Day.—Embryo three-quarters the length of egg; eye crescents distinct; *pleuropodia distinct*; *abdominal spiracles present*; coronal suture indistinct; epistomal suture prominent; Fig. 11.
- 12-Day.—Embryo fills egg; eye half pigmented; *hind femora three-quarters length of abdomen*, *beginning of chevron pigmentation*; pronotum distinct; Fig. 12.
- 13-Day.—*Dorsal closure incomplete on terminal three to four segments*; light chevron pigmentation on hind femora; Fig. 13.
- 14-Day.—Eye almost fully pigmented; *pigmented denta on mandibles*; dorsal closure complete; Fig. 14.
- 15-Day.—*Tibio-tarsal spurs black*; 'peppered' pigmentation on body surface; Fig. 15.

<sup>4</sup>Phrases in italics indicate those characteristics which are most easily used to identify the age of the embryo.

*16-Day.*—*Tibial spines black*; distinct pigment bands on hind femora; Fig. 16.

*17-Day.*—*Femoro-tibial joint darkly pigmented*; body fully pigmented; ready to hatch; Fig. 17.

Although embryogenesis was completed in 17 days at 30° C, this period was shortened to 15 days at 35° C. However, at 25° C hatching commenced 24 days after oviposition. This not only corresponds precisely with the embryonic developmental period of the diapause form of *M. bilituratus* (6), but also indicates that the embryogenesis of the nondiapause form proceeds 'normally'. This makes it an ideal type for the study of various aspects of the life history of the species in the embryonic stage, because such studies are not beset with the tedium of the diapause waiting period. Work is underway at present on the genetics of this form and on other related aspects of embryogenesis.

#### Acknowledgment

The writer wishes to thank his colleague, Mr. Roy Pickford, Canada Agriculture Research Station, Saskatoon, Saskatchewan, who initiated the selection and rearing of the nondiapause form of *M. bilituratus* and permitted the use of this material in the present study.

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## BIOLOGY AND TAXONOMY OF CEREAL AND GRASS APHIDS IN NEW BRUNSWICK (HOMOPTERA: APHIDIDAE)<sup>1</sup>

G. B. ORLOB<sup>2</sup>

### Abstract

The occurrence, distribution, and biology of various cereal and grass aphids were noted in New Brunswick during 1960. Because of the similarity of *Rhopalosiphum padi* (L.) overwintering on Prunaeae, and *R. fitchii* (Sanderson) hibernating on Pomeae, summer forms of these aphids are referred to as the *R. padi-fitchii* complex. An aphid from *Phleum pratense* L., very similar and closely related to *Schizaphis graminum* (Rondani), is described as *S. graminum phlei* ssp. n. Circumstantial evidence indicates that early spring migrants of *Rhopalosiphum maidis* (Fitch) and *Macrosiphum avenae* (Fabricius) did not originate in the province.

### Introduction

The list of aphids infesting small grains and grasses is not very long, but each species deserves special attention because of the role it may play as virus vector. Thus a study of the biology and taxonomy of cereal and grass aphids seemed likely to yield some background information on the role of aphids as transmitters of barley yellow dwarf virus. Emphasis was placed on observing aphids in their natural habitat, for this has the advantage of associating the insect with its host plant, and also indicates the distribution and abundance of aphids. Two kinds of aphid traps were used to supplement field observations (17).

### *Rhopalosiphum padi-fitchii* Complex

#### Taxonomy

In recent years considerable confusion has prevailed as to the taxonomic status and the synonymy of the olive-brown aphid so common in grain fields of the temperate zone. Workers in North America have long preferred the name *Rhopalosiphum fitchii* (Sanderson) or apple grain aphid, but *R. padi* (L.) or oat bird-cherry aphid has recently been widely accepted. The reason for this inconsistency is the apparent inability to distinguish summer forms of the two aphids, which, however, are easily separable on their respective winter hosts.

Richards has recently treated the genus *Rhopalosiphum* in a comprehensive monograph (19). He recognized *R. fitchii* and *R. padi* as distinct species, but states that the summer viviparae are easily confused and are only distinguishable by the slightly longer antennae and shorter, blunt antennal hairs in *R. padi*. That Richards rarely found summer forms of *R. fitchii* is surprising, for this aphid is rather common in many areas on the winter hosts.

Two comparable *Rhopalosiphum* species with a Rosaceae-Gramineae host cycle occur in Europe. Rogerson has examined spring migrants of *R. fitchii*

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collected from apple in Colorado, U.S.A., and considered them identical with European *R. insertum* (Walk.) (21). Hille Ris Lambers, in an attempt to clear up the confusion surrounding this group, arrived at a similar conclusion in stating that the North American *R. fitchii* is the same as the European *R. insertum*, and that the North American *R. prunifoliae* (Fitch) is identical with the European *R. padi* (9). Richards in Canada has described a new species, *R. viridis* Richards, as morphologically inseparable from *R. insertum* of European workers (19).

In the present study, fundatrigeniae of the two *Rhopalosiphum* species were collected from their respective winter hosts and subsequently transferred to barley. After small colonies had been established by the progeny of these aphids, alate and apterous forms were examined for morphological differences, but none were found. In accordance with Richards (19), both species were alike in their feeding site on grain, and the observations of Rogerson (20) and Hille Ris Lambers (9) that *R. insertum* (= *R. fitchii*) is subterranean in habit could not be confirmed. Search for *Rhopalosiphum*-like aphids on subterranean parts of grains and grasses in the field was also unsuccessful.

It is therefore concluded that the *padi-fitchii* (*insertum*) group needs further study before its taxonomic and biological status is resolved. Until these problems have been worked out satisfactorily, it seems advisable to refer to aphids of this group found on the summer hosts as the *R. padi-fitchii* complex.

### Biology

In the Fredericton area, fundatrices of both *R. padi* and *R. fitchii* hatched in the last week of April; fundatrices appeared around May 15, 1960; the first spring migrants were seen on the winter hosts on May 23, and a few were found in oat fields and in wind traps around May 30. *Rhopalosiphum padi* was most common on *Prunus virginiana* L., though smaller numbers were present on *P. pensylvanica* L. and *P. nigra* Ait. The apple grain aphid was less abundant on its winter hosts, *Malus* sp. and *Crateagus* sp.

Some field experiments were made with the fundatrices and their progeny by cross-transferring each of these forms of the two *Rhopalosiphum* aphids from their respective winter hosts to those of the related species. Both aphids were found to be host-specific within their host range, and had disappeared from the tree buds within 2 days after transfer to the host of the related species. Later, on May 26, a few fundatrigeniae of *R. padi* were found on *Crataegus* sp. mixed in colonies of *R. fitchii*. Obviously these aphids had reached this plant from nearby stands of *P. virginiana*, which were colonized extensively by *R. padi*.

As has been observed earlier, fundatrices and fundatrigeniae in the early instar stages do not adapt to a grain host (15). Despite this tendency, three fundatrigeniae, which were deposited on barley by fundatrices, could be reared in the present studies until they molted into spring migrants. These aphids seemed so well adapted to the unusual host that they could be repeatedly transferred to other barley plants without being kept in a feeding cage.

Throughout summer, *R. padi-fitchii* was the most common aphid on oats, which is the major small-grain crop in New Brunswick. Adams and Mac-

Gillivray have made similar observations during previous years (1, 10). After a considerable flight activity was noted in experimental plots on June 13 and 14, the peak of aphid migration occurred from June 22 to July 1.

Aphids of the *padi-fitchii* group were also found on various grasses, but colonies remained smaller on these hosts (18). With the ripening of grain, the usual population decrease developed and aphids remained scarce until September when numerous sexuales were present on the winter hosts.

#### ***Rhopalosiphum maidis* (Fitch)**

The corn leaf aphid was among the first of the aphids to be noticed. Spring migrants and several nymphs were found on *Agropyron* sp. between May 9 and May 15. With the exception of a few isolated areas around Fredericton, the aphid was rather scarce in New Brunswick's barley fields. *Rhopalosiphum maidis* was seen on several grasses that have been reported as host plants; in addition it was found on *Lolium perenne* L., and MacGillivray (11) reports collection from *Agrimonia* sp. and *Phaseolus* sp.

#### ***Macrosiphum avenae* (Fabricius)**

According to Adams (1) and MacGillivray (10), *M. avenae* in New Brunswick never reaches the population level of *R. padi-fitchii*. During the present season, the English grain aphid—although present in many grain fields—was not common, except in a few fields where numerous aphids fed on the heads of maturing oats. Spring migrants were first noticed on May 10, depositing a few nymphs on *Agropyron repens* L. and *Dactylis glomerata* L. The first specimen was trapped on June 2. No evidence for overwintering of this aphid in the province could be obtained. The English grain aphid belongs to the group of cereal aphids in so far as it is less prevalent on grasses.

#### ***Metopolophium dirhodum* (Walker)**

This aphid appeared on barley around June 15, but was less common than all other cereal and grass aphids observed. *Rosa* sp. and also *Fragaria* sp. and *Agrimonia* sp. have been reported as winter hosts, Gramineae and *Iris* sp. as summer hosts (7). In New Brunswick, MacGillivray has found oviparae and males on *Rosa* sp. (10). In at least one instance, oviparae and eggs have been reported to appear on barley in the greenhouse (16). These findings could be repeated in the present work, and in addition large numbers of males were produced. Hille Ris Lambers has compared our species with European material, and found them the same. Therefore, the possibility must be ruled out that in the past and present studies *M. dirhodum* has been confused with closely related nonmigratory species as they have been reported from Europe (7, 16).

The production of sexuales under greenhouse conditions does not necessarily imply holocyclic overwintering on Gramineae in nature. Theobald, in England, considered a hibernation of this aphid on grain possible, because he found it on cereals as early as April (23). On the other hand, Börner presented evidence that *M. dirhodum* and several other species do not overwinter on the summer host, but are established each year by being passively carried across the North Sea (2).

*Schizaphis graminum phlei* ssp. N.

During May, a *Schizaphis*-like aphid appeared on *Phleum pratense* L. in the Fredericton area. Feeding habit, damage inflicted to the host, as well as morphological characters suggested that this aphid was nothing but the well-known greenbug, *Schizaphis graminum* (Rondani). Other features, such as a yellow color and the presence of exclusively apterous forms early in the season, were not considered typical for *S. graminum* s. str. Soon it became apparent that the aphid from timothy differed also from the greenbug in its host range and the production of sexuales. Because of these characters the aphid is described as a new subspecies of *S. graminum*.

*Description**Apterous Viviparous Females*

Color in life: yellow to pale yellowish, rarely with a green tinge. Faint longitudinal streaks medially and laterally, but not as distinct as in *S. graminum* s. str. Cornicle yellow with dusky tip. Antennae dusky beyond base of III segment. Legs pale. Tarsi, apices of tibiae slightly darker.

*Morphology*.—Frontal tubercles well developed, exceeding median tubercle. Antennae shorter than body, six-segmented, without secondary rhinaria. Processus terminalis 3 to 3½ times the base of VI segment. Antennal hairs pointed, less than half the basal diameter of III segment. Rostrum short,

TABLE I  
Measurements of *Schizaphis graminum phlei* in mm

Form*	Body length	Antenna	Tibia	Cauda	Cornicle
Apterae viviparae					
1	1.54	1.012	0.574	0.161	0.233
2	1.25	0.924	0.503	—	0.182
3	1.40	0.881	0.486	—	0.167
4	1.30	1.069	0.591	0.133	0.219
5	1.34	0.868	0.473	—	0.174
6	1.31	0.948	0.467	—	0.166
Alatae viviparae					
7	1.51	1.124	0.662	0.113	0.148
8	1.47	1.188	0.645	0.127	0.154
Oviparae					
9	1.61	0.994	0.574	0.179	0.227
10	1.69	0.860	0.604	0.176	0.201
11	1.58	0.979	0.495	—	0.206
12	1.54	0.935	0.446	0.134	0.203
13	1.76	0.969	0.548	0.153	0.201
Males					
14	1.22	1.339	0.684	0.089	0.127
15	1.21	1.388	0.667	0.099	0.139
16	1.20	1.323	0.671	0.079	0.129
17	1.25	1.412	0.706	0.093	0.125
18	1.18	1.361	0.664	0.096	0.133

\*DATA: 1, field, Fredericton, N.B.; 2-18, greenhouse, Fredericton, N.B.

usually extending to middle coxae, last segment  $\frac{3}{4}$  to  $\frac{4}{5}$  as long as second joint of hind tarsi. Prothorax with well-developed lateral tubercles. Lateral tubercles present at least on abdominal segment I and VII. Setae on abdominal terga short, usually less than basal diameter of III antennal segment. Ventral abdominal hairs exceeding basal diameter of III segment. Cornicle longer than cauda (Table I), cylindrical in shape, imbricated without a distinct flange. Cauda bearing five hairs. Setae on hind tibia mostly longer than its apical diameter. For measurements see Table I.

Mounted specimens seem to be indistinguishable from those of *S. graminum* s. str.

#### *Alate Viviparous Females*

Color in life: essentially as in apterous females, but with head and pleural area brownish.

**Morphology.**—Antennae six-segmented, segment III with two to five rhinaria, none on segment IV. Processus terminalis about  $3\frac{3}{4}$  times as long as base of segment VI. Wings with media once forked. Cauda with four hairs. Otherwise as in apterous females.

Two mounted specimens appeared inseparable from alate *S. graminum* s. str.

#### *Oviparous Females*

Color in life: as in apterous females, but often more brownish.

**Morphology.**—Hind tibiae slightly to moderately swollen, bearing from 11 to 41 flat sensoria. Otherwise as in apterous viviparous females.

#### *Alate Males*

Color in life: as in alate females.

**Morphology.**—Shorter than alate females (Table I). Antennae longer than body, segment III bearing from 14 to 19 rhinaria; segment IV, 7 to 10; segment V, 4 to 8. Cauda with four or five hairs. Otherwise as in alate females.

#### *Type Material*

Holotypes and type locality: Apterous viviparous female, June 1, 1960. Fredericton, N.B., on *Phleum pratense*, to be stored in the Canadian National Collection. Paratypes: in author's collection and Canadian National Collection.

#### *Biology*

On *Phleum pratense* this aphid lives in a habit similar to *S. graminum* s. str. Dull brown necrotic spots appear on the leaves after short feeding periods. In the greenhouse, large colonies caused considerable damage to timothy, the infested leaves turning brown and finally dying. In the field, populations remained much smaller, and some time had to be spent before aphids could be located. Timothy grown for forage was most liable to attack, probably because the tender growth after cutting was suitable for aphid build-up. Oviparous females and males appeared in September, and were particularly abundant when the insect was reared in the greenhouse without supplemental light. Although no fundatrices were identified, the life cycle of the aphid suggests holocyclic overwintering on timothy.

*Biological Comparison between S. graminum s. str. and S. graminum phlei ssp. n.*

Because no morphological differences were found between *S. graminum* s. str. and *S. graminum phlei* special attention has been given to distinguishing the two aphids by biological characters. Stock colonies of *S. graminum* s. str. originated from Wisconsin, U.S.A.

*Host range.*—On timothy in the greenhouse, rapid parthenogenic reproduction of *S. graminum phlei* takes place, but this grass seems hardly acceptable to *S. graminum* s. str. When 20 timothy plants were infested three times weekly with numerous greenbugs, only eight nymphs were deposited on the host; a few aphids were still present 3 days after transfer, while the great majority dispersed quickly. Oats, on the other hand, is a most suitable host for *S. graminum* s. str., but not for *S. graminum phlei*, which fed for only a short period and died within 3 days when confined by means of cages to the oat plants. Oviparous females, of *S. graminum phlei*, however, could be maintained on oats for more than 10 days and seemed better adapted to this host. No grass other than timothy was colonized by *S. graminum phlei* under greenhouse conditions.

*Production of sexuales.*—In the fall of 1960, colonies of *S. graminum* s. str. and *S. graminum phlei* were maintained in the greenhouse under similar conditions and the production of sexuales observed. On September 15, most of *S. graminum phlei* had developed into oviparae and males, while no sexuales were present among colonies of *S. graminum*. Three weeks later, only oviparae and males were found of *S. graminum phlei*, but neither form was seen among the greenbug colonies.

*Comments*

Several *Schizaphis graminum*-like aphids have been reported previously. Hille Ris Lambers described *S. agrostis* H.R.L. from *Agrostis* sp., and *S. holci* H.R.L. from *Holcus* sp. (8). Eastop, in England, had treated these two aphids as subspecies of *S. graminum* (4). Another species, *S. jaroslavi* (Mordvilko), has been found in Russia on *Calamagrostis epigeios* (22). In Sweden two aphids of the *S. graminum* complex are present, one on *Agrostis* sp. similar to *S. agrostis*, and one on *Phleum pratense* (14). The latter aphid seemed indistinguishable from *S. graminum* except for its six or seven caudal hairs, and it was therefore taken as *S. graminum*, since the greater number of caudal hairs could have been due to the climatic conditions (14). More recently Tambs Lyche has identified another related species as *S. borealis* Tambs Lyche, which occurred in Norway on timothy (22). It can be separated from the greenbug by the shorter cornicles, which in *S. borealis* are of the same length as the cauda.

The timothy aphid found in New Brunswick did not differ significantly from *S. graminum* s. str. in morphological characters, but could easily be separated by its yellow color and some biological features. Possibly one may assume that *S. graminum phlei* has been derived from the mainly anholocyclic *S. graminum* by a holocyclic adaptation to a common grass and by this means has become established in an area which is rarely invaded by the greenbug.

***Sipha agropyrella* Hille Ris Lambers**

Fundatrices of the aphid were first found on May 9, and colonies on quack grass were frequently encountered thereafter. An account of this aphid has been published by MacGillivray (12).

***Hyalopteroides humilis* (Walker)**

This species has been described in detail by Hille Ris Lambers from Europe (7). A record from North America was given by Essig (6). Since Hille Ris Lambers did not find oviparae, this form will be described below.

**Description*****Oviparous Females***

Color in life: pale yellow to yellow. All appendages except hind tibiae and tips of tarsi colorless.

**Morphology.**—Much as in viviparous females. Body elongated and narrow, 1.474–2.057 mm. Frontal tubercles exceeding flat median tubercle. Antennae entirely pale, III segment with two to four rhinaria. Processus terminalis 3 to  $3\frac{1}{2}$  times the base of VI segment. Antennal hairs less than half the diameter of III segment. Length of antennae 1.078–1.358 mm. Rostrum short not reaching middle coxae, apical segment slightly longer than half the length of the second tarsal joint. Cauda rather long, 0.1931–0.2198 mm, not constricted, bearing seven hairs. Cornicle short, 0.0633–0.089 mm, coniform, with distinct imbrications. Basal half of hind tibiae slightly swollen with numerous sensoria.

**Biology**

The aphid was found on *Dactylis glomerata* from May 10 until early October. *Hyalopteroides humilis* can be rather common in some localities, although it was not regularly encountered wherever the host plant grew. The aphids feed on the upper side of the leaves, usually several insects lined up in a row, and the species could often be detected by the feeding damage caused on orchard grass. No alatae were found on the host, but a few were collected in aphid traps. Males and oviparae developed in late September, and could also be produced by rearing aphids in the greenhouse. *Hyalopteroides humilis* seemed to be strictly monophagous and could not be established on any one of the grasses and small grains tested.

**Where Do New Brunswick's Grain Aphids Come From?**

Workers differ in their opinion as to the origin of the spring population of aphids. Following the pioneering observations of Börner (2) and Elton (5), it has been repeatedly suggested that aphids may cover great distances by being passively carried in the aeroplankton. In the opinion of other workers, the spring population in temperate climates such as northern Europe and North America is entirely derived from the local population that overwinters holocyclicly or survives the winter anholocyclicly on the summer hosts.

Under suitable climatic conditions, grain aphids have been shown to overwinter anholocyclicly in such numbers as to multiply quickly in early spring (3). In New Brunswick, where winters are severe, anholocyclic overwintering seems rather unlikely as has been demonstrated for a comparable region of the continent (17).

Holocyclic overwintering of *R. padi-fitchii* has been reported from many areas and it is well established that every spring, migrants from the winter host initiate the local population on grain. But it was with this species that evidence of an introduction of aphids from outside areas has been obtained. At a time when the local population had developed fundatrices on the winter host, alatae of the same species were already found on the summer hosts (17).

With species such as *R. maidis*, *S. graminum*, *M. avenae*, where overwintering in the egg stage has not been reported or appears to be rare, long-distance translocation may be of greater importance in the invasion of an unoccupied area. Several observations have been made on the dispersal of the greenbug from its hibernating places in the southern United States into northern states and Canada (13, 24). Apparently suitable weather conditions of short duration suffice for the establishment of aphids in new territories (17).

At Fredericton, alate *R. maidis* and *M. avenae* were seen early in May. At this time of the season, no mature spring migrants had been produced by any of the local grass or cereal aphids. Fundatrices of *R. padi-fitchii*, which is one of the earliest hatching aphids, were approaching maturity, while fundatrices of aphids overwintering on grasses were just hatching or present as first instars. If the early migrants of *R. maidis* and *M. avenae* had been produced by a local population, an unusually speedy development of these aphids must be postulated in addition to an anholocyclic or holocyclic overwintering for which no evidence has been obtained in New Brunswick.

A few apterous or alatoid instars of *R. maidis* and *M. avenae* were also seen early in May, either alone in groups of two or three aphids or more frequently together with spring migrants.

High-altitude weather conditions prevailing from May 8 to 14 were found suitable for the arrival of aphids in the province, but too little is known at the present time to connect weather data properly with the dispersal of aphids. A beginning has just been made in the comprehension of the many factors involved in the long-distance translocation of small insects. Besides conditions governing the dispersal of aphids, areas of take-off and population development in these regions need study in the form of co-operative research.

Although indirect evidence seems to indicate long-distance transport of aphids into the province, there is little doubt that it is of less importance in New Brunswick than in other parts of the continent such as the north-central United States and adjacent Canadian territory. This is suggested by the fact that in New Brunswick *R. padi-fitchii*, known to overwinter in the area, is regularly the most common species and appearance of this aphid on grain almost coincides with its departure from the winter host. Other species which apparently do not spend the winter in the area are much scarcer at this time of the year, probably because relatively few migrants are blown in from outside areas.

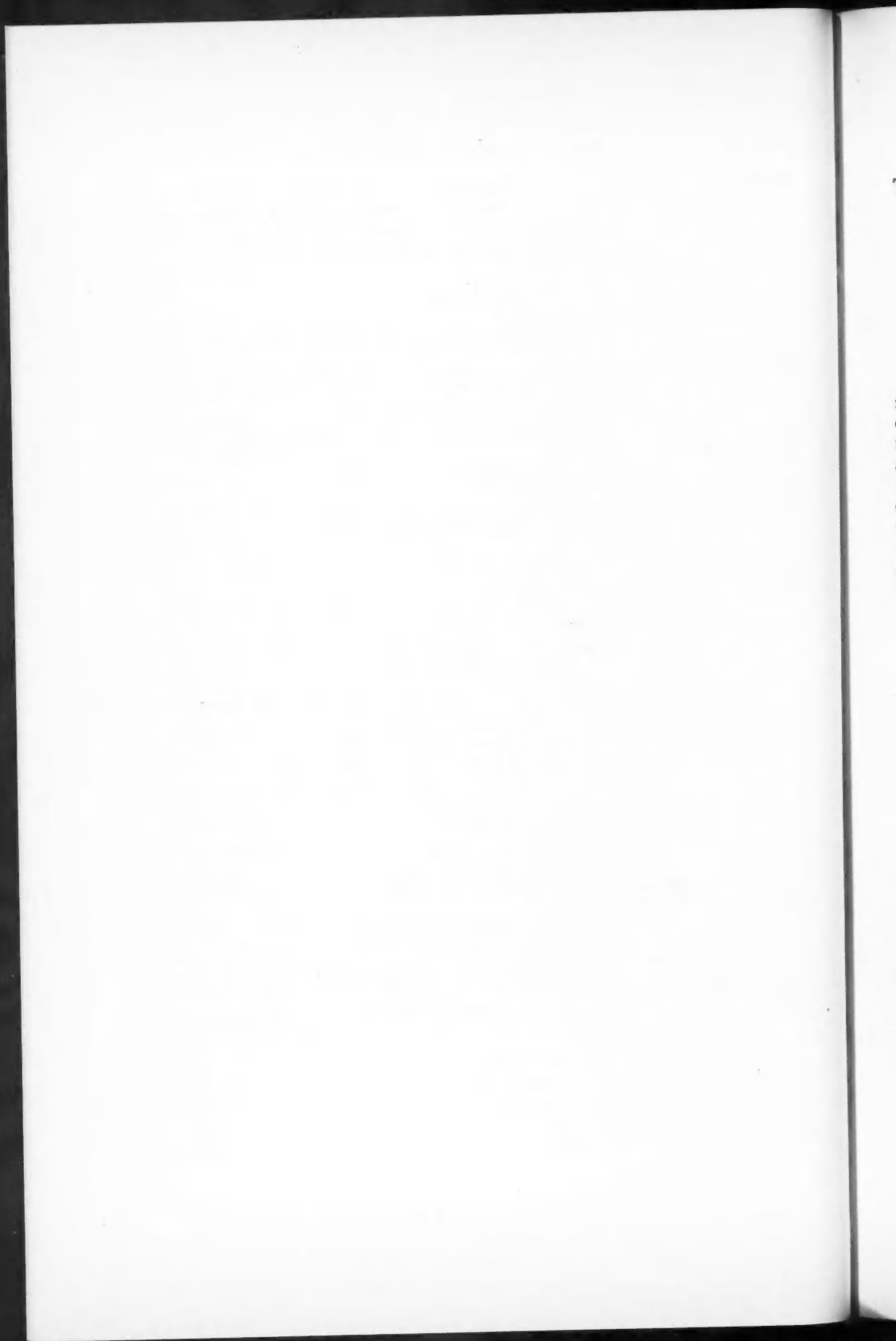
#### Acknowledgments

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especially Dr. V. F. Eastop, British Museum (Natural History), London. I am indebted to Mrs. J. B. Adams and Dr. M. E. MacGillivray, Canada Department of Agriculture, Fredericton, N.B., for helpful information. Stock colonies of *S. graminum* were kindly supplied by Dr. W. F. Rochow, Cornell University, Ithaca, N.Y.

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## TWO NEW SPECIES OF THE GENUS *HELICOTYLENCHUS* STEINER, 1945 (NEMATODA: HOPLOLAIMINAE)<sup>1</sup>

MUHAMMED WASEEM

### Abstract

*Helicotylenchus canadensis* n. sp., and *H. cairnsi* n. sp., commonly associated with red clover (*Trifolium pratense* L.) root zone in the province of Quebec, Canada, are described herein.

A survey of the plant parasitic nematodes associated with the root zone of red clover (*Trifolium pratense* L.) was conducted in the Province of Quebec during the summers of 1958 and 1960. Soil and root samples were collected from a total of 41 locations. Separation of nematodes from the samples was accomplished by utilizing a modification of the Christie and Perry technique (2). Of the plant parasitic nematodes recovered by this method, specimens of the genus *Helicotylenchus* Steiner, 1945 (9), were encountered most frequently. These specimens were found to represent at least three species: *H. digonicus* Perry, 1959 (in Perry *et al.* (8)), and at least two undescribed species. This paper deals with two new species which are herein referred to as *Helicotylenchus canadensis* n. sp., and *Helicotylenchus cairnsi* n. sp.

### *Helicotylenchus canadensis* n. sp.

(Figs. 1-4)

*Holotype*.—Hermaphrodite: Canada, Quebec, Experimental Station, Ste. Anne de la Pocatière. 12. IX. 1958 (M. Waseem). Host plant: *Trifolium pratense* L. Canadian National Collection of Nematodes, Ottawa, Canada, No. 2436. Fixed in TAF—see Courtney *et al.* (4)—and mounted in glycerin—see Baker (1).

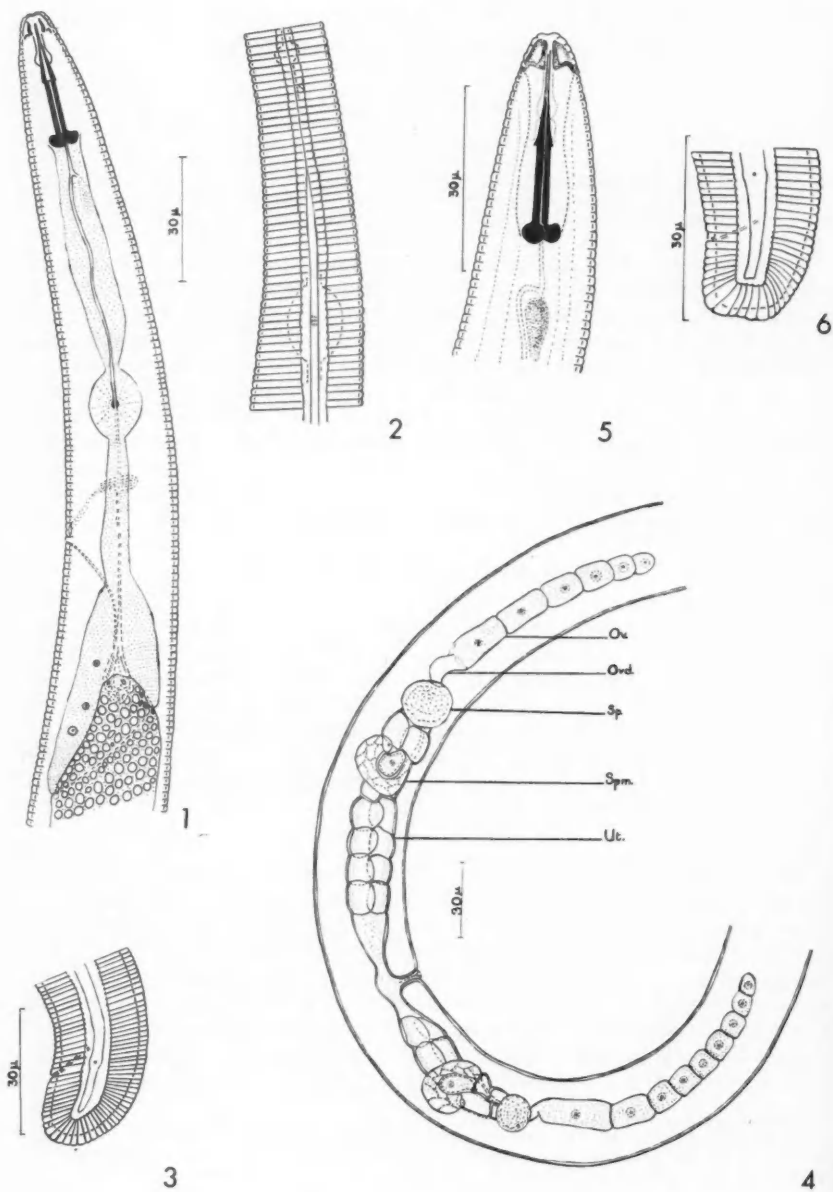
*Paratypes*.—Fifteen hermaphrodites: Same data as holotype. Canadian National Collection of Nematodes, Ottawa, Canada, No. 2436A. Fixed and mounted in TAF.

### Description

Body tapering anteriorly and posteriorly forming an open spiral when relaxed. Head not offset, lip region divisible into a concave labial annule, and four postlabial annules. The annules close to the neck region and those on the lip region somewhat narrower than those on the remainder of the body. There are six lips on which 14 papillae are discernible in *en face* view. The amphidial openings located near the outer margin of the lateral lips are often invisible. Cuticle and subcuticle transversely striated. The transverse striae are interrupted laterally by four incisures which, excepting the oesophageal region, occupy one-fourth of the body width; beginning of the lateral fields and

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subsequent separations forming a 'nonaerolated' third ribbon (Fig. 2), as reported for *Rotylenchus buxophilus*<sup>2</sup> by Golden (5). Widening of the lateral field in the vulvar and phasmidial region distinct. Phasmids dotlike, generally located in the anal region in mature specimens.

The dorsally convex tail (Fig. 3) is rather short, approximately 12–16  $\mu$  long, ventrally bearing 10–12 annules.

Stylet massive with well-developed anteriorly concave basal knobs; spear tip and spear shaft measuring approximately 14 and 16  $\mu$  respectively. Opening of the dorsal oesophageal gland 6 to 9  $\mu$  from base of stylet knobs.

Median bulb ovate, possessing a conspicuous valvular apparatus. Nerve ring located pre-equatorially on the isthmus. Lobelike portion of oesophagus highly variable in shape, overlapping the anterior portion of intestine.

Digonic hermaphrodite, spermatogonium, spermatheca, ovary, and oviduct distinct (Fig. 4) in the live specimens examined 12 hours after immersion in 0.1% aqueous solution of gentian violet. Ovaries paired outstretched, anterior arm 40–45  $\mu$  longer than the posterior arm. Oöcytes arranged in a single file; double oöcytes not seen. One-celled egg measuring 76  $\times$  23  $\mu$  while still in the uterus.

#### Measurements

*Holotype*.—de Man formula:<sup>3</sup>  $L = 0.86$  mm;  $a = 24.5$ ;  $b = 5.2$ ;  $c = 62.3$ ;  $V = 2964^{24}$ .

Cobb formula:<sup>4</sup>  $\frac{3.9 \quad 14.0 \quad 17.4 \quad 2964^{24} \quad 98.4}{2.1 \quad 3.1 \quad 3.1 \quad 4.1 \quad 2.3} \quad 0.86$  mm.  
Stylet = 30  $\mu$  long;  $o = 25$ .<sup>5</sup>

*Paratypes* (means of 15 individuals).— $L = 0.78$  (0.68–0.97) mm;  
 $a = 24.3$  (20.0–30.4);  $b = 5.4$  (4.8–6.7);  $c = 56.4$  (48.7–65.0);  
 $V = 64$  (61–66)%.  
Stylet = 30 (28–30)  $\mu$  long;  $o = 26$  (21–29).

#### Diagnosis

*Helicotylenchus canadensis* closely resembles *H. digonicus* Perry, but differs from it in having a distinct spermatheca; distance of dorsal gland orifice from the base of stylet knobs; tail shape, number of annules on the tail; total length; number of annules on lip region, and the shape of the labial annule.

<sup>2</sup>Now *Helicotylenchus buxophilus* (Golden, 1956) Perry, 1959 (in Perry *et al.* (8)).

<sup>3</sup>de Man, 1876 (7); see also Goodey, 1957 (6).

<sup>4</sup>Cobb, 1913 (3). In a future paper the author intends to discuss the merits of Cobb's formula and to demonstrate its usefulness in nematode taxonomy. It is because the author believes in its superiority over the formula of de Man (7) that it is included in the present work.

<sup>5</sup>Distance in microns from the stylet knobs to the dorsal gland outlet  $\times 100$  expressed as percentage of length of stylet in microns; cf. Perry *et al.* (8).

FIGS. 1–4. *Helicotylenchus canadensis* n. sp. Fig. 1. Anterior region. Fig. 2. Beginning of the lateral fields and subsequent separations. Fig. 3. Tail. Fig. 4. Reproductive system (abbreviations: Ov., ovary; Ovd., oviduct; Sp., spermatheca; Spm., spermatogonium; Ut., uterus).

FIGS. 5–6. *Helicotylenchus cairnsi* n. sp. Fig. 5. Head. Fig. 6. Tail.

***Helicotylenchus cairnsi* n. sp.**

(Figs. 5-6)

**Holotype.**—Hermaphrodite: Canada, Quebec, Experimental Station, Ste. Anne de la Pocatière. 12. IX. 1958 (M. Waseem). Host plant: *Trifolium pratense* L. Canadian National Collection of Nematodes, Ottawa, Canada, No. 2437. Fixed in TAF and mounted in glycerin.

**Paratypes.**—Fifteen hermaphrodites: Same data as holotype. Canadian National Collection of Nematodes, Ottawa, Canada, No. 2437A. Fixed and mounted in TAF.

**Description**

Body tapering anteriorly and posteriorly forming a more or less closed spiral when relaxed by gentle heat. Head continuous with body contour except for an almost imperceptible constriction in cephalic region. Lip region with a labial annule and four postlabial annules. There are six lips on which 14 papillae are discernible in *en face* view. Amphidial openings located near the outer margin of lateral lips. Cuticle and subcuticle transversely striated; two cuticular annules equal to five subcuticular annules. Transverse striae are interrupted laterally by four incisures which occupy one-fourth of the body diameter. Lateral incisures terminating as shown in Fig. 6. Phasmids dotlike, variable in position, located 8-10 annules anterior to anus in mature individuals.

Tail distinctive, short, truncate, approximately 12-14  $\mu$  long, ventrally bearing 7-9 annules; terminal annule widened. Tail with only slight dorsal curvature.

Stylet massive with well-developed rounded basal knobs which appear anteriorly concave in certain lateral views. Spear tip and spear shaft 15 and 17  $\mu$  long, respectively. Distance of dorsal gland orifice from stylet base is approximately 6 to 9  $\mu$ , and always less than one-third stylet length. Median oesophageal bulb ovate with a conspicuous valvular apparatus. Nerve ring located near the base of median bulb; lobelike portion of oesophagus variable in shape overlapping the anterior portion of intestine; only three gland nuclei seen. Junction of oesophagus and intestine rather obscure.

Vulva a depressed transverse slit with small lateral flaps. Digonic hermaphrodite with general description of gonads as for *H. canadensis*.

**Measurements**

**Holotype.**—de Man formula:  $L = 0.79$  mm;  $a = 24.8$ ;  $b = 5.8$ ;  $c = 61.1$ ;  $V = 3165^{23}$ .

Cobb formula:  $\frac{4.1 \quad 16.2 \quad 17.1 \quad 3165^{23} \quad 98.5}{2.1 \quad 3.1 \quad 3.2 \quad 4.0 \quad 2.1} \quad 0.79 \text{ mm.}$   
Stylet = 32  $\mu$  long;  $o = 25$ .

**Paratypes** (means of 15 individuals).— $L = 0.76$  (0.71-0.85) mm;  
 $a = 24.2$  (21.9-27.8);  $b = 5.8$  (4.9-7.0);  $c = 58.1$  (48.0-66.4);  
 $V = 63$  (60-65)%  
Stylet = 30 (28-32)  $\mu$  long;  $o = 25$ .

### Diagnosis

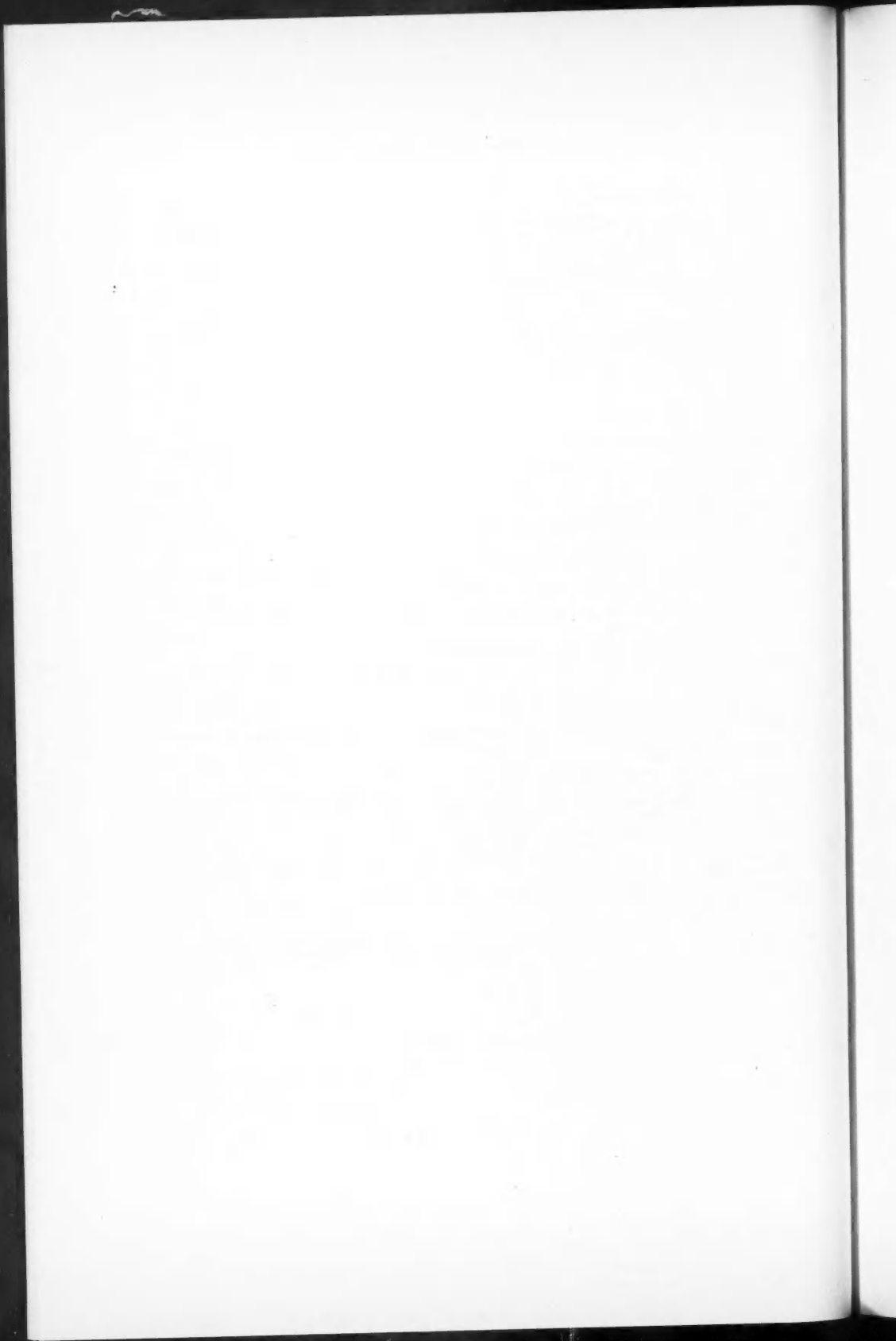
*Helicotylenchus cairnsi* can be distinguished from all known digonic hermaphrodite species of *Helicotylenchus* by the short truncate tail. *Helicotylenchus cairnsi* most closely resembles *H. platyurus* Perry, but can be distinguished from the latter species by the following characteristics: *H. cairnsi* has 7-9 annules on tail, whereas *H. platyurus* has 15 annules. In *H. cairnsi* the phasmids are placed rather forward of anus, whereas in *H. platyurus* they are located in the anal region. The distance of the dorsal gland orifice in the species *H. cairnsi* is about seven microns from the stylet base, whereas this distance in the species *H. platyurus* is about twelve microns. The vulva in the specimens of *H. cairnsi* is much farther removed from the anterior end than in *H. platyurus*.

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## HISTOPHYSIOLOGICAL STUDIES ON THE JUVENILE ATLANTIC SALMON (*SALMO SALAR*) RETINA

### II. RESPONSES TO LIGHT INTENSITIES, WAVELENGTHS, TEMPERATURES, AND CONTINUOUS LIGHT OR DARK<sup>1</sup>

M. A. ALI<sup>2</sup>

#### Abstract

Retinal epithelial pigment dark-adapts in intensities below  $10^{-3}$  ft-c. Cones are in a light-adapted state in intensities of  $10^{-3}$  ft-c and above, semiadapted state in  $10^{-3}$  ft-c, and dark-adapted state in  $10^{-4}$  ft-c and below. The retinal index corresponds to the response of cones. It is suggested that the visible spectrum of the yearling Atlantic salmon ranges from 3640 Å to 6900 Å because the retina is in a light-adapted state in that range. In light, lower and higher temperatures bring about an expansion of the retinal pigment. Cones and retinal index are not affected. In dark, pigment expands with higher temperatures but temperatures exceeding 18.3° C bring forth contraction. Cones contract with increasing temperatures but beyond 14.8° C they expand. The retinal index indicates a greater dark-adaptation of retina with increasing temperatures between 5.0° C and 18.3° C but higher temperatures make it less dark-adapted. Cones exhibit no rhythm in their positions in continuous light or dark. Pigment demonstrates no rhythm in light but shows one in dark which persists for a day. The significance of the results is discussed. Comparisons with results with Pacific salmon are made.

#### I. Introduction

In the first paper of this series (6) the rates of light- and dark-adaptation have been presented. In this paper the photomechanical responses of the juvenile Atlantic salmon retina to various light intensities, wavelengths, temperatures, continuous light, and continuous dark will be described.

The significance of the retinal responses to various light intensities has been discussed earlier (1). Retinomotor and photobehavioral responses have been correlated in the case of some species and stages of the Pacific salmon (3, 5).

In an investigation of the visual physiology of any animal, one of the important and interesting aspects is the ascertaining of the range of its visible spectrum. An animal may or may not be capable of perceiving colors. However, regardless of whether it can differentiate among the various wavelengths as most humans do, it has a visible spectrum ranging from one particular wavelength to another. Within this range, it is able to perceive light either as different hues or as various shades of gray.

Teleosts as a group, with the possible exception of deep sea forms with pure rod retinæ, are believed to be able to perceive colors (20). Walls (20) has also discussed the investigations undertaken by several workers, prior to 1942, in order to ascertain whether some teleosts can discriminate among colors. All these workers employed behavioral methods in their experiments.

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In most instances light intensity appears to have been an additional variable due to the use of colored papers and bulbs. It is well known that when light of a particular intensity is shone on papers of various colors, the intensity of the light reflected by these papers varies from one to the other. Similarly, bulbs of the same wattage but of different colors produce lights that differ considerably in their intensities.

Studies with the absorption spectra of the retinal pigments from various fishes have provided considerable information concerning the visible spectra of fish. Papers by Wald (19) and Munz (16) and the recent review of Crescitelli (13) provide further details and bibliographies. The use of the electrophysiological technique to obtain the visible spectra of fish appears to be gaining ground (13).

As far as I am aware no one has attempted to obtain the visible spectrum of any fish using histological methods, although several workers have studied their retinomotor responses (1). In this paper, the results of experiments conducted in order to obtain the visible spectrum of the yearling Atlantic salmon are also presented. These experiments were undertaken on the premise that, if under certain wavelengths of light the eyes of the experimental animals were in a light-adapted state, then evidently the fish could see the light of those wavelengths. If on the other hand, their eyes were in a dark-adapted state under certain other wavelengths, then obviously they were not able to perceive light of those wavelengths.

The positions of the retinal elements of certain fishes and amphibians are known to be influenced by temperature (2, 8, 14). When this histophysiological study of the juvenile Atlantic salmon retina was undertaken, it was felt that an investigation aimed at ascertaining the influence of temperature on the retina, in light and in dark, would be essential if we were to evaluate the results of an experiment wherein temperature happens to be an additional variable, often because of unavoidable reasons.

The retinal elements of certain fishes exhibit a diurnal rhythm in their positions under continuous light or continuous dark (18). Generally, this rhythm is more pronounced in dark and is very faint or totally absent under constant light (9, 21, 22). In three species and three stages (alevin, late fry, and smolt) of the Pacific salmon no rhythm was exhibited by the retinal pigment or the cones either in continuous light or in continuous dark (1).

In subsequent studies with the yearling Atlantic salmon it is hoped to study the retinal responses using electrophysiological methods and also to investigate the behavioral responses such as feeding and pseudorheotropism, at various light intensities, wavelengths, and temperatures with a view to ascertaining whether there is any correlation among the results obtained using histophysiological, electrophysiological, and behavioral means.

## II. Material and Methods

### A. MATERIAL

Yearlings obtained from the Margaree Hatchery, Frizzleton, N.S., of the Canada Department of Fisheries were used. In the Fish Research Laboratory of Memorial University, they were kept in aerated and refrigerated running

water. The amount of light entering the fish room varied with the duration and intensity of natural light. The fish were fed daily on a diet of ground raw beef liver. Their lengths ranged from 5.3 cm to 8.5 cm.

## B. METHODS

### 1. *Light Intensities*

These experiments were carried out in July, 1960, and were conducted during the forenoons, between 9 a.m. and 12 noon. Light intensities of  $10^2$ ,  $10^1$ ,  $10^0$ ,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  ft-c were created using filament bulbs ranging from 2.5 to 60 watts and by varying their distance from the experimental tank ( $30 \times 24 \times 24$  cm). Light intensities of  $10^{-1}$  ft-c and over were measured using a Photovolt model 200-M photometer. Intensities lower than these were measured with a Photovolt model 514-M photometer equipped with a special filter in order to obtain uniform spectral sensitivity between 3000 Å and 7000 Å.

The aquaria in which fish were exposed to the different light intensities were supplied with running water kept at  $15^\circ \pm 1^\circ$  C during these experiments. The fish were exposed in groups of five to each of the light intensities mentioned above for 2 hours, and they were then fixed in standard Bouin's solution.

### 2. *Wavelengths*

Experiments were conducted during the months of July and August, 1960. They were carried out in the mornings between 9 a.m. and 12 noon. Monochromatic filters of 3060, 3385, 3640, 3900, 4200, 4500, 4800, 5100, 5400, 5700, 6000, 6300, 6600, 6900, 7200, and 7500 Å, purchased from Messers Barr and Stroud of Glasgow, Scotland, were used with a Leitz projector as a light source, in order to create lights of various wavelengths. Light intensity was kept constant at 60 ft-c. This was accomplished by altering the distance between the light source and the aquarium ( $30 \times 24 \times 24$  cm) in the light-proof experimental room and by using apertures of various diameters. The light intensities were measured with the Photovolt model 514-M photometer mentioned above. The temperature of the running water in the aquaria varied from  $18^\circ$  C to  $21^\circ$  C during the period in which these experiments were conducted. The fish were exposed in groups of five to each of the wavelengths for 2 hours and they were then fixed in Bouin's solution.

### 3. *Temperatures*

Experiments were carried out during the latter part of July, 1960. They were conducted only in the forenoons (9 a.m. to 12 noon). Five fish were kept at each of the temperatures shown (Fig. 3) for 2 hours in light. Similarly, groups of five fish were kept at each of the temperatures (Fig. 3) for 2 hours, but in dark. All were fixed in Bouin's fixative.

### 4. *Continuous Light and Continuous Dark*

The experiments were performed in June, 1960. A hundred fish were placed in tanks with running water ( $15^\circ$  C) either in constant light (25 ft-c) or in constant dark. A sample of five fish were removed from each tank every 6 hours (12 a.m., 6 a.m., 12 p.m., and 6 p.m.) for 96 hours (4 days). Neither the group in light nor the group in dark was fed during the experiment.

### 5. General

The histological technique employed, method of measurement of thicknesses of retinal layers, and the calculation of retinal index were similar to those described in an earlier paper (6). The retinal index, which is calculated from the thicknesses of the cone and pigment layer, reduces the variations which occur as a result of individual differences in the thicknesses of the retinae, pigment, and cone layers. Further, it makes it possible to consider the responses of the pigment and cone layers as a single response. Each point in the figures (Figs. 1-4) is the mean of 50 measurements made from 10 eyes.

## III. Results

### A. RESPONSE TO DIFFERENT LIGHT INTENSITIES

The retinal epithelial pigment was in a light-adapted state in the eyes of fish exposed to  $10^2$ ,  $10^1$ ,  $10^0$ ,  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  ft-c. It was in a dark-adapted state in fish exposed to  $10^{-4}$  and  $10^{-5}$  ft-c (Fig. 1).

The cone layer was in a light-adapted (contracted) state in the retinae of fish exposed to  $10^2$ ,  $10^1$ ,  $10^0$ ,  $10^{-1}$ , and  $10^{-2}$  ft-c, in a semiadapted state for  $10^{-3}$  ft-c, and in a dark-adapted state for  $10^{-4}$  and  $10^{-5}$  ft-c (Fig. 1).

The retinal index indicates the commencement of dark-adaptation when the intensity decreases below  $10^{-1}$  ft-c and its completion when the light

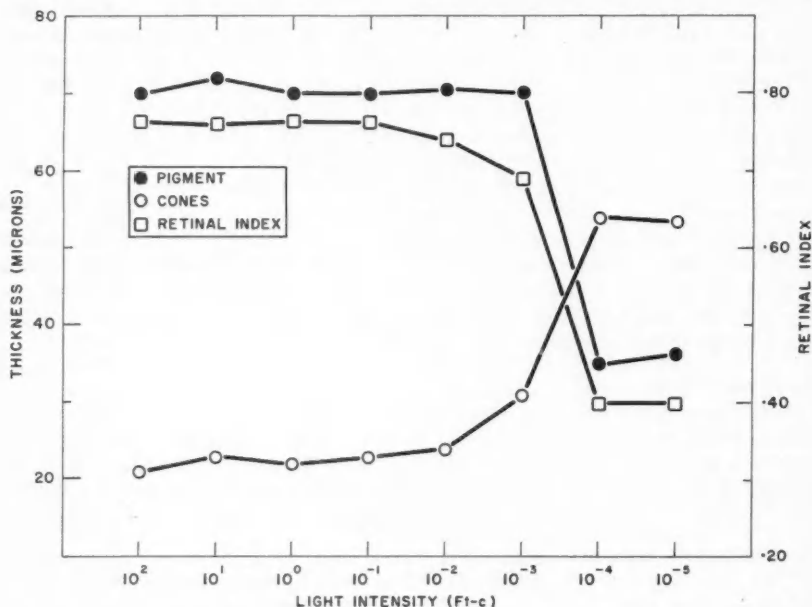


FIG. 1. Graph showing the thicknesses of the retinal epithelial pigment and cone layers in the eyes of fish exposed to various light intensities. The calculated retinal indices are also shown.

intensity is  $10^{-4}$  ft-c or lower (Fig. 1). It may be assumed that it shows that the retina is in a fully light-adapted state at intensities of  $10^{-1}$  ft-c and above and in a dark-adapted state at intensities of  $10^{-4}$  ft-c or below.

#### B. RESPONSE TO DIFFERENT WAVELENGTHS OF LIGHT

In the eyes of fish exposed to light of wavelengths ranging from 3060 Å to 6900 Å, the retinal epithelial pigment was in a light-adapted state. It was in a dark-adapted state in the eyes of fish exposed to light of 7200 Å and 7500 Å (Fig. 2). Histological examination supported this observation. However, it is rather surprising that it (pigment) showed considerable variation in thickness in the retinae of fish exposed to light of 3060 Å to 6900 Å. The degree of variation shown here was considerably greater than that observed in the eyes of fish used in the other experiments of this and earlier investigations.

The cones, on the other hand, did not exhibit much variation in length and were in a light-adapted state in the retinae of fish exposed to light of wavelengths ranging from 3640 Å to 6900 Å (Fig. 2). In light of wavelength 3640 Å they are in a semiadapted state. At wavelengths of 3060, 7200, and 7500 Å they are in a dark-adapted state.

The retinal index shows a similar response to that shown by the cones, that is, it indicates that the retina is in a fully light-adapted state in fish exposed to light of wavelengths ranging from 3640 Å to 6900 Å. It also indicates that the retinae of fish exposed to wavelengths shorter than 3640 Å are in a semiadapted state and that those of fish exposed to wavelengths longer than 6900 Å are in a dark-adapted state (Fig. 2).

#### C. RESPONSE TO TEMPERATURE

##### 1. In Light (Fig. 3)

The retinae of fish kept at 2.0°, 5.7°, 11.5°, and 14.3° C demonstrate that the pigment is the more contracted the higher the temperature. On the other hand, temperatures of 18.0° and 21.5° C bring about an expansion of the pigment.

The measurements of cone layer thicknesses indicate that the cones do not respond to temperatures in light except possibly for the fish kept at 11.5° C, where a slight expansion is observed. This may perhaps be attributed to several eyes having thicker cone layers. It is known that in any sample of Atlantic salmon yearlings this can occur (4). However, the possibility of such an occurrence influencing the results may be discounted in this instance because the retinal index clearly shows the absence of any influence of temperature on the state of adaptation of the retina.

##### 2. In Dark (Fig. 3)

The thickness of the retinal epithelial pigment increases with higher temperatures, but when the temperature exceeds 18.3° C the effect is reversed and in the retinae of fish kept at 21.5° C a marked contraction is observed.

The cones contract with increase in temperature up to 14.8° C, after which further, higher temperatures cause an opposite effect, viz. expansion.

When the retinal indices of fish kept at various temperatures in dark are compared, it is seen that up to 5.0° C there is no change and after that, up

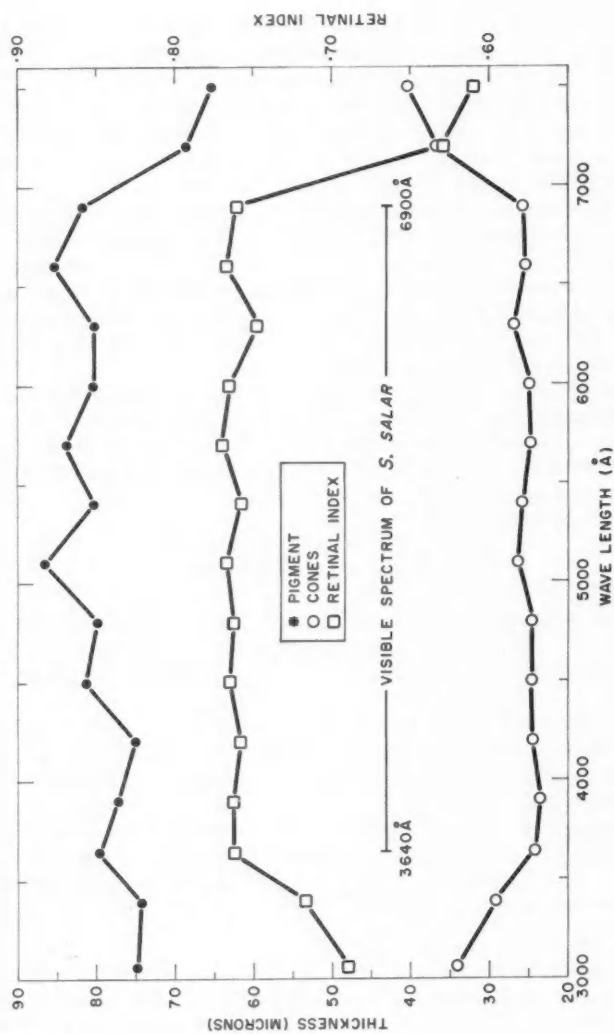


FIG. 2. Graph showing the state of retinal epithelial pigment, cones, and retinal index in the eyes of fish exposed to the various wavelengths mentioned.

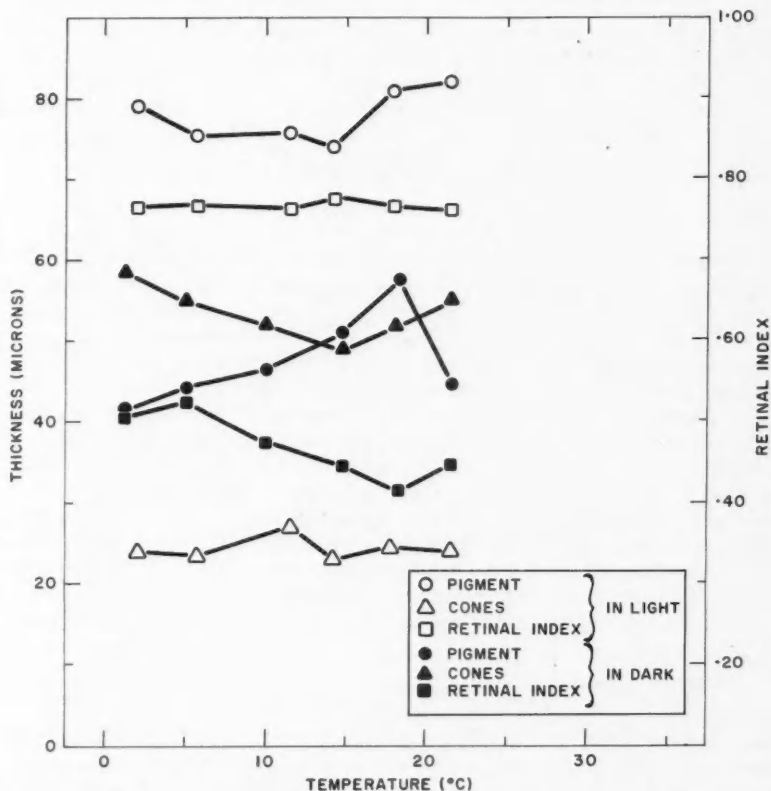


FIG. 3. Graph showing the thicknesses of retinal epithelial pigment and cone layers in light and in darkness at various temperatures. Retinal indices are also shown. The temperatures used were: 2.0°, 5.7°, 11.5°, 14.3°, 18.0°, and 21.5° C (in light); 1.2°, 5.0°, 10.0°, 14.8°, 18.3°, and 21.5° C (in dark).

to 18.3° C, the retinal index decreases, and then it increases when the temperature exceeds 18.3° C.

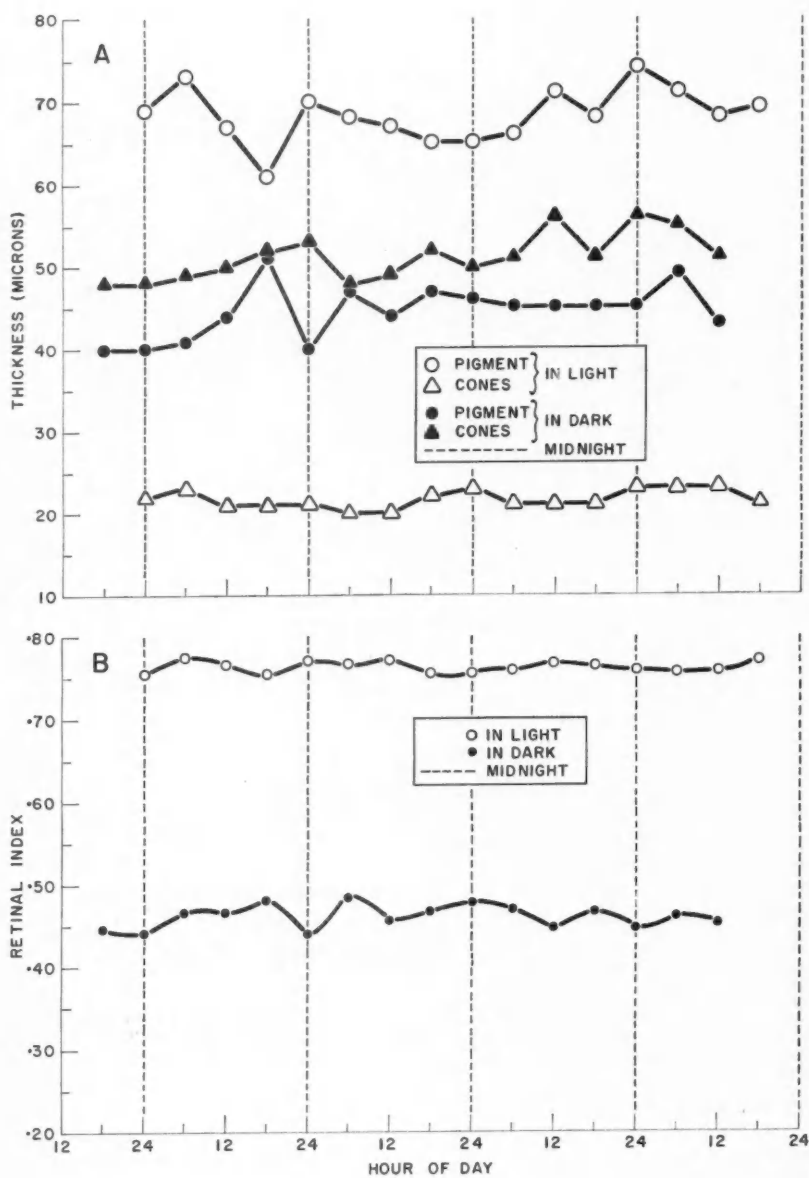
#### D. RESPONSE TO CONTINUOUS LIGHT OR DARK

##### 1. To Continuous Light (Fig. 4)

Neither the retinal epithelial pigment nor the cones possess a diurnal rhythm or indeed any rhythm in their positions in continuous light. Retinal indices show agreement with this observation.

##### 2. To Continuous Dark (Fig. 4)

In this case also the cone layer does not exhibit any rhythm. The pigment layer, however, appears to possess a diurnal (24-hour) rhythm in its position during the first day in dark. Histological examinations and the retinal indices support this observation.



The thickness of the pigment layer in the eyes of fish sampled at 6 p.m. on the first day is significantly different ( $p < 0.01$ ) from that of fish sampled at the first and second midnights. This rhythm persists only for a day, becoming irregular on the second day and virtually disappearing during the subsequent days.

#### IV. Discussion

##### A. GENERAL

##### 1. Light Intensities

The responses of the retinal pigment and the cones to various light intensities are interesting in themselves because they show the morphological changes occurring within the retina. The calculation and use of the retinal index appears to serve a purpose in so far as it indicates what may be considered the combined photomechanical response of the pigment and cones. Evidently, experiments wherein electrophysiological and behavioral methods are employed will have to be carried out in order to ascertain whether there is any correlation between the retinal index and the "true" state of adaptation of the retina. In the case of late fry stages of pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon and the late fry and smolt stages of sockeye (*O. nerka*) and coho (*O. kisutch*) salmon the state of the cone layer has been correlated with their feeding response (1). Further, in the case of the pink (*O. gorbuscha*) late fry, the association between its downstream migration and state of adaptation of the retina has been shown (5). It is conceivable that similar correlations could also be obtained for the Atlantic salmon in future investigations.

##### 2. Wavelengths of Light

The variation exhibited by the retinal pigment thickness in fish exposed to light of wavelengths between 3060 Å and 6900 Å may partly be explained by its function. The Atlantic salmon, like most teleosts, lacks a contractile iris. Therefore, it appears to be the function of the retinal epithelial pigment to control the amount of light impinging on the cones. It may very well be that different wavelengths elicit different responses from the pigment because of differences in its absorption qualities. This variation is not seen in the pigment of fish exposed to 7200 Å and 7500 Å. It appears very likely that these wavelengths are not visible to the Atlantic salmon, hence, elicit the same response from the pigment and cones as darkness would. The retinal index also shows that the retina is in a fully dark-adapted state at these wavelengths.

In addition, it is necessary to recall that, in most investigations that I have undertaken with the retina (1, 5, 10), the retinal epithelial pigment invariably exhibited a greater variation than the cones or the rods did. Another factor responsible for this variation may have been temperature. The vari-

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FIG. 4. A. Thicknesses of retinal epithelial pigment and cone layers in the eyes of fish subjected to continuous light or to continuous darkness and sampled every 6 hours. The first complete 24-hour period (midnight to midnight) of the experiment is referred to as the first day in the text. B. Retinal index of the eyes of fish samples taken at various times. Here also the first complete 24-hour period of the experiment is considered the first day.

ation of 3° C observed during this experiment may have been, at least partly, responsible for bringing about changes in the thickness of the pigment. Temperature is known to affect the thickness of the pigment layer much more than it affects the cones (2). Experiments conducted during the course of this investigation have also shown that temperature does affect the thickness of the pigment layer in the yearling Atlantic salmon (Fig. 3). A further discussion of this aspect follows in a later section of this paper.

As has been pointed out earlier, in the case of the Pacific salmon there is a good correlation between the state of adaptation of their cones and their feeding and migratory responses (1, 5). Although experimental evidence is not yet available, it appears reasonable to assume that such a correlation can exist in the case of the Atlantic salmon also. It must be remembered that the fish used in this investigation are probably not in the migratory state. However, because of the fact that the cones are in a light-adapted state in the eyes of fish exposed to light of wavelengths ranging from 3640 Å to 6900 Å and that this corresponds to the curve yielded by the retinal index (Fig. 2), it may be suggested that the visible spectrum of the yearling Atlantic salmon ranges from 3640 Å to 6900 Å. Investigations using electrophysiological and photochemical methods and behavioral responses will have to be undertaken in order to ascertain whether there is any correlation among their results and those of the present investigation.

The suggestion that the visible spectrum of the yearling Atlantic salmon ranges from 3640 Å to 6900 Å implies that these fish are able to perceive light of these wavelengths. It does not imply that they are capable of discriminating among these wavelengths. They may very well possess this capacity but it is not within the scope of this investigation to suggest this.

### 3. *Temperatures*

The comparatively smaller effect of temperature on the retinal pigment in light and the lack of response of cones and retinal index to temperature in the presence of light is interesting. It may be taken to indicate that, in the presence of light, which is the primary stimulus in so far as the retina is concerned, secondary factors, such as temperature, have no influence, or, at best, have only a small influence. Another postulation that may be made, based on these and the results of an earlier investigation (2), is that the difference in the response of the pigment and the cones to temperature in the presence of light may be due to the fact that the former is nonnervous while the latter is nervous. While the pigment may be controlled by hormones (intermedin?) alone, the cones, being part of the sensory retina, may, to a large extent if not totally, be under neural influence. It will be worth while studying this aspect for, although some studies along these lines have been made (7, 17), our knowledge concerning these factors is scarce.

It is also necessary to discuss the results of the other experiments of this investigation, in the light of those obtained in the temperature experiments. It would appear that the rates of light- and dark-adaption (6) will not have been affected by temperature because during the 70 minutes the experiment lasted, the temperature did not change. In the case of retinal responses to light intensities (Fig. 1) also the influence of temperature may be ruled out

since during that experiment it was  $15 \pm 1^\circ \text{C}$  and within this range the magnitude of its influence on pigment and cone positions was not sufficient to affect the results significantly. In the experiments with different wavelengths (Fig. 2), however, it appears probable that temperature may have played some part in altering the results. These experiments were conducted during a hot spell when water temperature rose and ranged between  $18^\circ \text{C}$  and  $21^\circ \text{C}$ . It has been pointed out earlier (Fig. 3) how temperatures within this range can affect the retinal epithelial pigment (in light and in dark) and the cones (in dark only). For the sake of this comparison, the range  $3640 \text{ \AA}$  to  $6900 \text{ \AA}$  (visible spectrum of salmon) must be assumed to be equivalent to the light condition and wavelengths shorter than  $3640 \text{ \AA}$  and longer than  $6900 \text{ \AA}$  must be considered dark, in terms of the experiment with different temperatures. Notable effects may have been obtained for the pigment at wavelengths shorter than  $3640 \text{ \AA}$  and longer than  $6900 \text{ \AA}$  but not within the range of the visible spectrum ( $3640 \text{ \AA}$  to  $6900 \text{ \AA}$ ) because the pigment in the eyes of fish kept at  $18^\circ \text{C}$  and  $21.5^\circ \text{C}$  in light does not show much difference between the two groups (Fig. 3). The cones would not have been influenced by temperatures ranging from  $18^\circ \text{C}$  to  $21^\circ \text{C}$  within the visible spectrum, but beyond it, may have been affected. Figure 3 shows that cones undergo slight contraction at temperatures higher than  $18.3^\circ \text{C}$ , in dark. It may be postulated that the reason for the cones being in a semiadapted state in the eyes of fish exposed to wavelengths shorter than  $3640 \text{ \AA}$  (Fig. 2) may be due to the fact that the temperature of water on the days these experiments were conducted was  $21^\circ \text{C}$ . This suggests strongly that strict temperature control would be a valuable addition in further experiments.

During the experiments with continuous light and continuous dark (Fig. 4) the temperature of the water remained constant at  $15^\circ \text{C}$ ; hence the possibility that the temperature had altered the results can be discounted.

#### 4. Continuous Light or Dark

##### (a) Continuous Light

It is necessary to discuss certain interesting features of the results. While there is no significant difference among the thicknesses of the cone layers of fish samples taken at different times, the pigment layers of fish samples taken during the first 24 hours show considerable variation in thickness. It was observed that, where the difference between average thicknesses of two groups of pigment thicknesses was considerable, there was also a statistically significant difference between the two groups of thicknesses. For example, when the thicknesses of the pigment layers of fish samples taken at 6 a.m. on the first day are compared with those of fish samples taken at 6 p.m. on the same day, the difference is very significant ( $p < 0.01$ ). On the other hand, there is no significant difference among the thicknesses of pigment layers of fish samples taken during the second day and the early part of the third day. As can be seen from the graph (Fig. 4A) the pigment layers of fish samples taken during the latter part of the third day and during the fourth day show some variation in thickness but this variation, unlike the one on the first day, is not statistically significant ( $p < 0.2$ ). Observations made by careful histological examination of the sections show that where the average thickness

of the pigment is great (e.g. 6 a.m., first day) the pigment is fully dispersed in the epithelial cell processes and almost touches the external limiting membrane. In a situation where the average thickness of the pigment layer is smaller (e.g. 6 p.m., first day) it is not fully expanded but is withdrawn into the epithelial cell processes to the extent that cone outer segments can be seen.

This variation exhibited by the pigment layer in continuous light cannot be considered as being due to an inherent rhythm for two reasons. First the variation shown by the thickness of the pigment does not follow a rhythmic pattern, that is, it is not in a less expanded state at midnight and in a fully expanded state during the day. Indeed, it is in a fully expanded state at 6 p.m. on the first day, in the least expanded state at 6 a.m. on the first day, and in a moderately expanded state at the first two midnights of the experiment. Had there been a rhythm, the pigment would have been in the least expanded state at the midnights and in a fully expanded state during the day. Secondly, the retinal index, which eliminates variations, does not indicate any rhythm.

It is suggested that, light being an active stimulus, the variation in the thicknesses of pigment layers of fish samples taken during the first day is a result of the fishes' attempts to get acclimatized to this continuous stimulus. The virtual lack of variation in the thicknesses of the pigment layers in the eyes of fish samples taken during the second and early third day may appear to be due to their having adapted themselves to this continuous stimulus. The variation which reappears during the third (last) phase of the experiment may be due to the constant light becoming a stress after the fish have been exposed to it for more than 60 hours. A condition of stress, of course, would upset the hormonal balance, which in turn would affect the dispersal of pigment. The foregoing suggestion will have to be substantiated by further physiological studies.

#### *(b) Continuous Dark*

The visual elements of certain fish are known to exhibit a diurnal rhythm in their positions in dark, although it is lacking in light (17, 21, 22). This appears to be the case in the yearling Atlantic salmon. Despite the variation exhibited by the thicknesses of the cone layers in dark it is not possible to label it a diurnal rhythm because it does not follow a definite pattern. In the eyes of the fish samples taken the first midnight (Fig. 4A) they are in a less expanded state, and in the eyes of the fish samples taken 24 hours later they are in an almost fully expanded state. In the samples taken at 6 a.m. on the second day they are in a less expanded state which is similar to that shown by the cone layers of fish samples taken the first midnight.

The pigment layer, however, shows a pattern that may be called a diurnal rhythm. The thicknesses of the pigment layers of fish samples taken the first and second midnights are in the most contracted state and during the first day the pigment gradually increases in thickness, attaining the maximum thickness (in the dark-adapted state) at 6 p.m. As has been pointed out earlier, the thicknesses of the pigment layers of fish samples taken the first two midnights are significantly different ( $p < 0.01$ ) from those of fish samples taken at 6 p.m. on the first day. In view of this, it appears safe to assume that

the retinal epithelial pigment of the yearling Atlantic salmon exhibits a diurnal rhythm in its position in continuous dark. That it persists only for a day may mean that the yearling Atlantic salmon is not a very rhythmic animal. For the purposes of this discussion, a rhythmic animal may be defined as one in which, under constant external environmental conditions, the internal body functions follow a diurnal or seasonal rhythm. In general, the more rhythmic the animals are, the greater will be the period of persistence of a rhythm in constant conditions.

The very first points in the graphs (Fig. 4) showing the data in continuous dark at 6 p.m. differ significantly ( $p < 0.01$ ) from those showing the condition at the next 6 p.m. (first day). This raises a question as to why there should be such a difference between two sets of data showing the states of the retinal pigment, cones, and the retinal index in fish samples taken at the same time of the day (viz. 6 p.m.) during the first 24-hour period of the experiment. It may be that by placing the fish in darkness at noon the normal rhythm was upset temporarily.

Another point warrants mention here. Both the pigment and cone layers of fish samples taken during the last 48 hours of the experiment have an average thickness that is higher than those of the fish samples taken during the first 48 hours of the experiment. Since both the pigment and cone layers become thicker the retinal index does not show any such difference. Perhaps even a passive stimulus such as darkness, when fish are left in it for extended periods, becomes a condition of stress after several hours. The fact that these fish as well as those in continuous light were not fed during the course of the experiment (4 days) may have been a factor also. It seems desirable to conduct investigations to ascertain the effect of continuous light or continuous dark on metabolic rates, etc.

## B. COMPARISONS

### 1. General

Before undertaking a comparison of these results with those obtained in previous investigations with some stages and species of the Pacific salmon (1, 2), the differences in life histories and behavior patterns between the two genera should be borne in mind. The five species of the Pacific salmon occurring on the west coast of Canada differ among themselves in so far as their life histories and behavior patterns are concerned (11, 12).

Information is available concerning the retinal responses of sockeye (alevin, late fry, and smolts), coho (late fry and smolts), pink (late fry), and chum (alevins and late fry) salmon to light intensities. Retinal responses of sockeye (alevins, late fry, and smolts), coho (late fry), and chum (alevins and late fry) to continuous light and to continuous darkness are known (1). Only in the case of sockeye late fry has the effect of temperature on the retina been studied (2). Responses to different wavelengths have not been studied in any stage or species of the Pacific salmon.

### 2. Response to Light Intensities

Experiments with different light intensities indicate that the retina of the yearling Atlantic salmon has a much lower threshold than the retina of any other species or stage of the Pacific salmon.

The retinal epithelial pigment of the Pacific salmon commenced dark-adapting at a much higher intensity:  $10^0$  ft-c in chum, pink, and coho late fry and coho smolt, and  $10^1$  ft-c in sockeye late fry and smolt. It is also interesting to note that except in the case of chum late fry, the retinal epithelial pigment of all the other species and stages studied was in a semicontracted state under some light intensities ( $10^{-1}$ ,  $10^{-2}$  ft-c). This has not happened in this investigation with the Atlantic salmon (Fig. 1). The pigment is in a light-adapted state under  $10^{-3}$  ft-c and in the next condition studied ( $10^{-4}$  ft-c) is in a dark-adapted state.

The cone layers of the four species of Pacific salmon studied (1) were also much less sensitive to light. The late fry of coho and the smolts of sockeye and coho possessed cones which were more sensitive and commenced dark-adaption when the intensity decreased below  $10^{-1}$  ft-c. The cones of the other species and stages remained light-adapted only as long as the light intensity was  $10^0$  ft-c or above.

It is also necessary to recall that only in the case of the sockeye late fry did the cones assume a semiadapted state ( $10^{-1}$  ft-c) while in all the others they were either light-adapted or dark-adapted. As has been mentioned earlier (Fig. 1), the cones of the yearling Atlantic salmon were in a semiadapted state at  $10^{-3}$  ft-c.

It is interesting that the cones of the coho salmon were more sensitive because based on migratory behavior it has been suggested that, of the five species of *Oncorhynchus*, it is the closest to the troutlike, parental type (15). Also, coho have a freshwater life history more similar to *S. salar* than to any other *Oncorhynchus* species.

In their yearling stage, the Atlantic salmon usually inhabit shallow streams and do not migrate downstream at dusk. Yearling salmon in the Little Codroy River in Newfoundland feed on small organisms and are themselves preyed upon by eels. They migrate during the day within the estuary. It may be argued that because of their sensitive eyes they are not subject to downstream displacement at dusk. A more acute vision would, of course, be an asset in capturing small organisms over a greater part of the day.

It is not possible, with the available information, to make any definite suggestion as to why the Atlantic salmon should have a more sensitive retina.

### 3. Effect of Temperature

The juvenile Atlantic salmon differs from the sockeye salmon (2) in the response of its retinal elements to temperatures in light as well as in dark. The cones of the sockeye were not influenced by temperatures ( $3-23^\circ\text{C}$ ) either in light or in dark while its pigment was affected by higher temperatures ( $15-23^\circ\text{C}$ ) in dark only. It is surprising that while the Atlantic salmon differed in this respect from its fellow salmonid, the response of its retina to temperatures, in light, was similar to that observed by Arey (8) using *Ameiurus*, *Fundulus*, *Abramis*, and *Carassius*, which are taxonomically further removed. However, the response of the Atlantic salmon retina to temperatures in dark was opposite to that of Arey's fishes.

The incompatibility of the results of the temperature experiments with those obtained with the sockeye salmon (2) may perhaps be explained, as in

the case of the rates of adaptation (6), as being due to the use of a lower light intensity (25 ft-c) in the former in comparison with the latter where a higher light intensity (400 ft-c) was used. Although only further investigations can elucidate this point, it does not appear probable that such profound differences will be obtained as a result of a difference in intensity alone. Had the general trend been the same but of a different magnitude (as was seen in the case of the rates of adaptation), it would lend greater support to the suggestion.

#### 4. Rhythms

None of the species or stages of the Pacific salmon studied in a previous investigation (1) showed any rhythm in the positions of the retinal epithelial pigment or cones. It was suggested that the absence of a diurnal rhythm in the positions of the retinal elements made it possible for the downstream migration to be related to the light intensity and not to the time of day.

That the retinal pigment of the yearling Atlantic salmon has a rhythm in continuous dark is not very significant from the point of view of its behavior or visual responses. The fact that the cones do not exhibit a rhythm is, however, significant because they are the receptors, the visual cells. This would indicate that the Atlantic salmon, like its fellow salmonid, the Pacific salmon, will respond to light conditions regardless of the time of day.

### V. Summary and Conclusions

1. The retinal epithelial pigment of the yearling Atlantic salmon remains in a light-adapted state until the light intensity decreases below  $10^{-3}$  ft-c. The cones are less sensitive and their threshold is  $10^{-2}$  ft-c. The retinal index shows the retina as such to be light-adapted at intensities of  $10^{-1}$  ft-c and above, semiadapted at  $10^{-2}$  ft-c, and dark-adapted at  $10^{-3}$  ft-c or below.

2. The retinal pigment and cones of the yearling Atlantic salmon are more sensitive than those of any of the species or stages of the Pacific salmon studied previously.

3. The pigment is light-adapted or semiadapted in fish exposed to 3060 Å to 6900 Å. It is dark-adapted in fish exposed to wavelengths longer than 6900 Å. The cones and retinal index indicate the retinae to be light-adapted at wavelengths of 3640 Å to 6900 Å, semiadapted at wavelengths shorter than 3640 Å, and dark-adapted at wavelengths longer than 6900 Å.

4. On the basis of these results, it is postulated that the visible spectrum of the yearling Atlantic salmon ranges from 3640 Å to 6900 Å.

5. The pigment is influenced by temperature in both light and dark while cones are affected only in dark. The retinal index shows the influence of temperature in darkness only.

6. The results of adaptation (6), light intensities, and continuous light or dark experiments do not appear to have been affected by temperatures but the wavelength experiment seems to have been influenced by higher temperatures. However, this has not diminished the significance of those findings.

7. Cones do not possess a rhythm in their positions in continuous light or dark. Pigment has no rhythm in light but shows a 24-hour rhythm in dark. This lasts for only a day. It is suggested that variations in pigment thickness occur when constant light or dark becomes a condition of stress.

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## CESTODES OF WOLVES, COYOTES, AND COYOTE-DOG HYBRIDS IN ONTARIO<sup>1</sup>

R. S. FREEMAN, A. ADORJAN, AND D. H. PIMLOTT

### Abstract

*Echinococcus granulosus*, *Taenia hydatigena*, and *T. krabbei* were the most common cestodes encountered in timber wolves of Ontario, with *T. pisiformis*, *T. laticollis*, and *T. crassiceps* being less common. *Taenia pisiformis* was the only common cestode of coyotes, although *T. hydatigena*, *T. laticollis*, *E. granulosus*, and *Mesocestoides* sp. were recovered. No *Multiceps* sp. was found. In Ontario, propagation of *T. pisiformis* apparently depends mainly on coyotes, whereas *E. granulosus*, *T. hydatigena*, and *T. krabbei* depend on wolves. *E. granulosus* was approximately twice as common in wolves from areas where moose are more common than deer, and conversely *T. hydatigena* and *T. krabbei* were approximately twice as common in wolves from areas where deer are more common than moose.

### Introduction

Parasitological examinations of 520 timber wolves (*Canis lupus* L.), 339 coyotes (*Canis latrans* Say), and 34 coyote-dog hybrids were made during a continuing province-wide study of the biology of these canids. Cestodes were found commonly, and those in a large sample were identified. This is the first survey of cestodes from these hosts in Canada, although the parasites of wolves and coyotes from Minnesota (3), of coyotes from Utah (1) and Kansas (4), and of wolves from Alaska (6) have been reported. The present data not only indicate the incidence and distribution of cestodes of wolves and coyotes in Ontario, but they reveal some aspects of the food habits of these hosts.

### Materials and Methods

The wolves and coyotes used in this study were collected November, 1957, through November, 1960. Most were frozen out of doors until they could be moved to the freezer at the central laboratory, but some were partly decomposed before examination. At autopsy the animals were weighed, measured, and the skull was saved for final identification. The gut was opened and scraped and intestinal parasites were collected without the aid of a microscope. The presence or absence of *Echinococcus granulosus* (Batsch, 1786) was recorded at this time; other cestodes when not too decomposed were preserved in 10% formalin for future identification. Whole mount preparations were made from a sample of the latter. Examination of the rostellar hooks proved the simplest and single most reliable method for identifying these worms. The rostellum was cut off the scolex and flattened under a cover-glass. If rostellar hooks were present the preparation was cleared and dehydrated in Beechwood creosote and if necessary mounted in Permout.

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Contribution from Department of Parasitology, Ontario Research Foundation, Toronto, Ontario (R.S.F.), and Research Branch, Ontario Department of Lands and Forests, Southern Research Station, Maple, Ontario (A.A. and D.H.F.). This work was made possible in part by a research grant to the Ontario Research Foundation by the Department of Commerce and Development, Province of Ontario.

Drawings of large and small rostellar hooks from metacestodes, as well as from adult worms, which were identified with certainty, were made to the same scale by means of a camera lucida. Direct comparison of all preparations with the appropriate drawings, by means of the camera lucida, identified all worms which had any rostellar hooks.

### Results

Cestodes occurred in 219 (42%) of the timber wolves, 140 (41%) of the coyotes, and eight (24%) of the hybrids. *Echinococcus granulosus* (Batsch, 1786) occurred in 103 (20%) of the wolves, 2 (0.5%) of the coyotes, and none of the hybrids. This parasite was recognized in heavy infections even when the host was in early stages of decomposition, although because of its small size it was probably overlooked in some light infections. Nevertheless, this gives a conservative estimate of the incidence of this parasite in these hosts in Ontario. Other cestodes which were identified from a sample of the positive animals were: *Taenia pisiformis* (Bloch, 1780); *T. hydatigena* Pallas, 1776; *T. krabbei* (Moniez, 1879); *T. laticollis* Rudolphi, 1819; *T. crassiceps* (Zeder, 1800); and *Mesocostoides* sp. Because the drawings of the large and small rostellar hooks were so helpful they are shown in Figs. 1 through 10. The rostellar hooks of *Taenia taeniaeformis* Batsch, 1786 and *Multiceps serialis* (Gervais, 1847)<sup>2</sup> from a mouse and rabbit from Ontario are also included, since they may be confused with some of the foregoing. The frequency of occurrence of the various cestodes is shown in Table I.

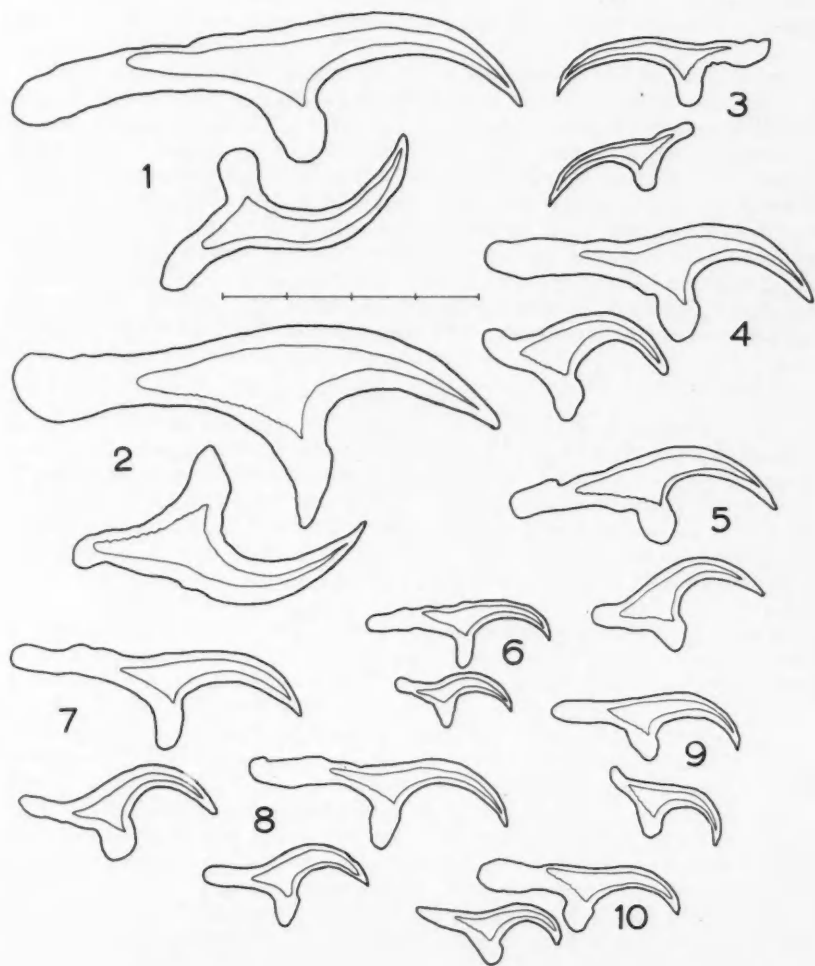
TABLE I

Incidence of cestodes other than *E. granulosus* in timber wolves, coyotes, and coyote-dog hybrids in Ontario

Host	Number where cestodes identified	Total with:					
		<i>Taenia pisiformis</i>	<i>Taenia hydatigena</i>	<i>Taenia krabbei</i>	<i>Taenia laticollis</i>	<i>Taenia crassiceps</i>	<i>Mesocostoides</i> sp.
Timber wolf ( <i>Canis lupus</i> )	58	7	39	17	1	1	0
Coyote ( <i>Canis latrans</i> )	68	66	3	0	1	0	1
Coyote-dog hybrid	6	6	0	0	0	1	0

*Taenia pisiformis* is obviously the only common cestode of coyotes in Ontario (Table I). *Echinococcus granulosus*, *T. hydatigena*, *T. laticollis*, and *Mesocostoides* sp. were recorded from coyotes seven times, the latter three cestodes occurring once each in conjunction with *T. pisiformis*. *Taenia hydatigena* and *E. granulosus* are the most common cestodes of wolves, followed by *T. krabbei*. *Taenia pisiformis*, *T. laticollis*, and *T. crassiceps* were found more rarely in wolves. Whereas multiple infections with cestodes occurred only three times in coyotes, 36 wolves had multiple infections. *Echinococcus granu-*

<sup>2</sup>There is confusion as to whether the genus *Multiceps* should be synonymized with *Taenia* and whether there is more than one species, viz. *multiceps*, *serialis*, and *packii*, of this complex present in North American canids and rabbits and hares. We consider that form found in Ontario is as indicated here.



FIGS. 1-10. Drawings of various rostellar hooks made to same magnification with the aid of a camera lucida. Each unit of scale equals 50 microns. Fig. 1. *Taenia laticollis*. Fig. 2. *Taenia taeniaeformis*. Fig. 3. *Taenia crassiceps*. Fig. 4. *Taenia pisiformis*, largest hooks encountered. Fig. 5. *Taenia pisiformis*, smallest hooks encountered. Fig. 6. *Multiceps serialis*. Fig. 7. *Taenia hydatigena*, largest hooks encountered. Fig. 8. *Taenia hydatigena*, smallest hooks encountered. Fig. 9. *Taenia krabbei*, smallest hooks encountered. Fig. 10. *Taenia krabbei*, largest hooks encountered.

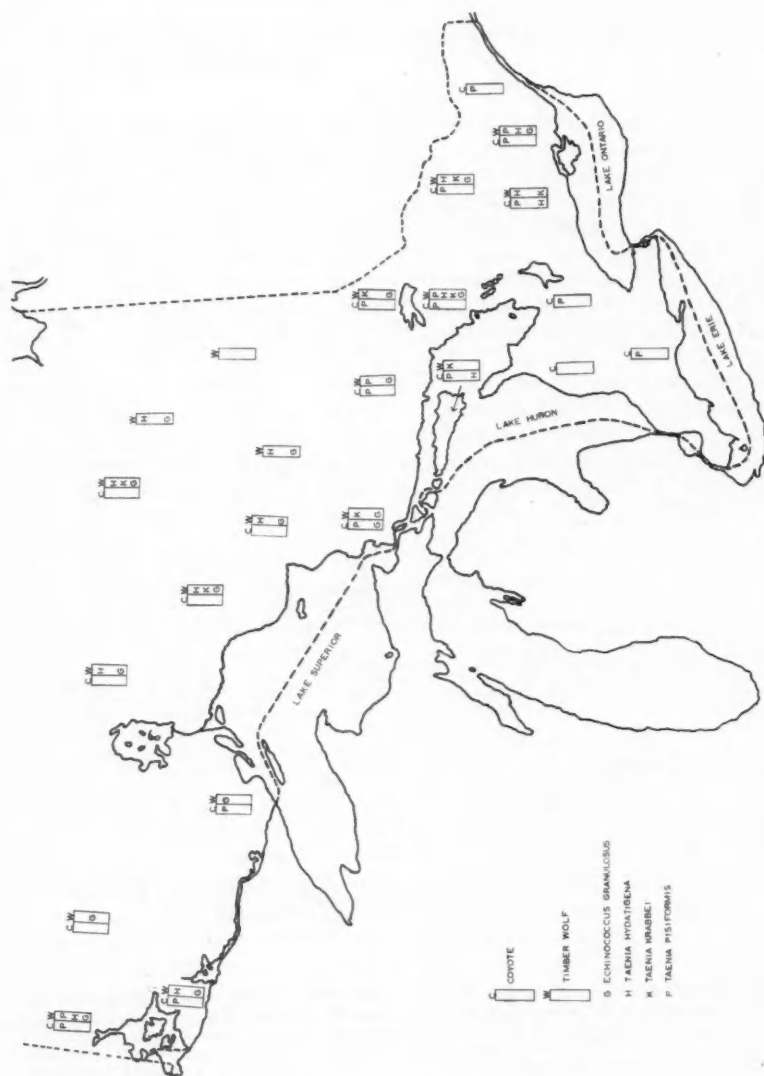


FIG. 11. Distribution of the four common cestodes of timber wolves and coyotes in the Province of Ontario.

*losus* occurred with another species of cestode in 32 wolves. Three species of cestodes were present in two wolves. Cestodes were less common in the coyote-dog hybrids than the other two hosts, and only *T. pisiformis* and *T. crassiceps* were found (Table I).

The distribution of *T. pisiformis*, *T. hydatigena*, *T. krabbei*, and *E. granulosus*, the four common species of cestodes of wolves or coyotes in this province, is shown in Fig. 11. *Taenia laticollis* occurred once in a timber wolf from northeast of Lake Superior (White River), and once in a coyote from due north of Georgian Bay (Sudbury). A timber wolf pup and a coyote infected with *T. crassiceps*, as well as almost all the hybrids infected with *T. pisiformis*, came from the area north of the eastern end of Lake Ontario (Tweed and Lindsay). The coyote with *Mesocestoides* sp. came from the Manitoulin Islands in northern Lake Huron. The incidence of the various cestodes in the different areas shown in Fig. 11 is not given owing to the small number of positive animals for most areas. The data on *E. granulosus* are more voluminous, however, and will be discussed more fully.

### Discussion

In Ontario, as elsewhere, the metacestode of *T. pisiformis* grows among the viscera of rabbits and hares. The metacestodes of *T. hydatigena*, *T. krabbei*, and *E. granulosus* develop mainly in the liver, musculature, and lungs respectively of moose and deer although *E. granulosus* is rare in the latter host. The metacestode of *T. crassiceps* grows subcutaneously in small rodents and was the common cestode we found in over 100 red foxes examined from Ontario (unpublished data). The life cycles of *T. laticollis* and *Mesocestoides* sp. are incompletely known.

The incidence of cestodes in coyotes and wolves suggests that coyotes in Ontario subsist heavily on rabbits and hares, and rarely on rodents and cervids (Table I). Infections of *T. hydatigena* in coyotes may result from scavenging wolf kills, as Cowan (2) observed in western Canada. The incidence of cestodes in coyote-dog hybrids was half that in wolves or coyotes, but the cestodes which were present indicate that they feed on rabbits and less frequently on rodents (Table I). Timber wolves subsist mainly on cervids and occasionally rabbits, taking rodents rarely in Ontario (Table I). The relatively few coyotes which had *T. hydatigena* and *E. granulosus* and the wolves which had *T. pisiformis* all occurred only where both coyotes and timber wolves along with their parasites are common. This suggests that the continued propagation of *T. pisiformis* depends largely on coyotes, and similarly propagation of *E. granulosus*, *T. hydatigena*, and *T. krabbei* depends on wolves in Ontario.

The incidence and distribution of *E. granulosus* in contrast to that of *T. hydatigena* and *T. krabbei* in wolves from different parts of Ontario was particularly interesting. In parts of the wolf range where deer are more common than moose, the two *Taenia* species were nearly twice as common as *E. granulosus*. Conversely *E. granulosus* was twice as common, on the average, as the two *Taenia* species where moose are more numerous than deer, except in northeastern Ontario around Swastika. Moose are common in this district, yet only three *Taenia* infections, which were not identified as to species,

and no *E. granulosus* were found in 29 wolves from this area. The over-all pattern of distribution of *E. granulosus* supports Sweatman (7), who maintained that *E. granulosus* in Ontario depends on the moose - timber wolf relationship.

Apparently *T. pisiformis* is the most common cestode of coyotes wherever this host occurs (1, 3, 4), although occurrence of this cestode was relatively more common in wolves from Minnesota (3) than from Ontario. *Taenia hydatigena* was not found in coyotes in Kansas (4), occurred rarely in this host in Utah (1), but was more common in coyotes from Minnesota (3) than Ontario. This cestode was common in wolves from Alaska (6) and Minnesota (3). *Taenia krabbei* also was common in Alaskan wolves (6), and, although absent from wolves in Minnesota, it occurred there in a coyote. This species was not reported from Utah or Kansas (1, 4). *Echinococcus granulosus* was reported previously from a coyote in Ontario (7) and one in Alberta (5), and it is common in wolves of Minnesota and Alaska (3, 6). We have seen the metacestode of *Multiceps serialis* in varying hares in Ontario, but have no record of it from canids in this province. It has been reported from coyotes and wolves elsewhere in North America (1, 3, 6).

#### Acknowledgments

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**SOME PREDATORS OF Aedes stimulans (WALK.)  
AND Aedes trichurus (DYAR) (DIPTERA:  
CULICIDAE) IN WOODLAND POOLS<sup>1</sup>**

H. G. JAMES

**Abstract**

The predators of first- and second-instar larvae of *Aedes stimulans* and *A. trichurus* in temporary woodland pools near Belleville, Ontario, were determined mainly by use of mosquito larvae tagged with radioactive phosphorus, but in part from the presence of aedine remains found in the digestive tracts of dissected beetles. Of 428 pond animals collected from the test pools 122 were found to be radioactive above background. Among the 17 species of aquatic insects and other animals that fed on mosquito larvae, 8 species of Dytiscidae, 1 of Hydrophilidae, 1 of Limnephilidae, and 1 pond snail are regarded as important predators. Three additional species of water beetles were identified as predators from aedine remains in their digestive tracts. The abundance of the predators, and the times of occurrence of six species in relation to mosquito development, are discussed.

**Introduction**

The role of predators in the natural control of mosquitoes was reviewed comprehensively by Hinman (8) in 1934. Among those who contributed field data, Hearle (7), Hintz (9), and Sailer and Lienk (11) pointed out that immature mosquitoes are less exposed to natural enemies in temporary than in permanent bodies of water. Comparatively little is known, however, of the relation of the predator fauna of transient pools to the species of Nearctic mosquitoes that breed in them.

Two of the most troublesome mosquitoes in southern Ontario are the brown wood mosquito, *Aedes stimulans* (Walk.), and *Aedes trichurus* (Dyar). These two species and their predators were investigated from 1955 to 1959, in temporary pools in a mixed hardwood swamp at Chatterton (Marsh Hill), Ontario. The term temporary, as used here, refers to vernal pools of 3½ months' duration that usually dry up by the end of June. The objectives of this study were to determine the identity and relative importance of the predators of the early-instar larvae, to estimate predator numbers, and to compare the seasonal occurrence and development of some of the principal predators with those of the pest species they attack.

**Materials and Methods**

In 1954, aquatic beetles were collected in the pools and their status as mosquito predators determined by dissection. This method can be used with species that ingest prey or parts of prey that have sclerotized structures that can be later identified. It is unsuitable, however, for detecting predators that have piercing or sucking mouth parts (Corixidae, Gerridae (Hemiptera)) or those with specialized mandibles, such as dytiscid larvae (4), that predigest prey. The limitations of this method were avoided in the predator studies of 1956 and 1958, by tagging mosquito larvae with radiophosphorus.

<sup>1</sup>Manuscript received May 3, 1961.

Contribution from Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario.

Radiophosphorus,  $P^{32}$ , as  $H_3P^{32}O_4$ , was obtained from Atomic Energy of Canada, Limited, Chalk River, Ontario. In preliminary tolerance tests, first-instar larvae of *Aedes aegypti* (L.) were exposed to the isotope in the laboratory at a concentration of  $0.1 \mu\text{c}/\text{ml}$  for 24 and 48 hours. Mean radiation counts of larvae 4 days later were 4,480 and 11,867 c.p.m. respectively. These treatments were not particularly injurious to the larvae, as more than 90% in each lot eventually pupated.

In the 1956 field experiment, a total of approximately 7,500 first-instar mosquito larvae were collected on April 19 and 20 from each of three pools that contained mixed populations of *A. stimulans* and *A. trichurus*. The larvae were transferred to 75 liters of strained pond water in plastic-coated metal tanks and were made radioactive by the method of Baldwin, James, and Welch (2), except that the concentration of the isotope was increased to  $0.1 \mu\text{c}/\text{ml}$ . After 48 hours they were washed twice in pond water and left 4 more days in fresh pond water to rid them of radioactive excreta. Ten of these larvae tested in the laboratory for radioactivity averaged 12,560 c.p.m. (range 5,800–19,500). Twenty-four hours after the larvae were returned to the test pools, aquatic insects and other pond animals were collected and taken to the laboratory and tested with a Nuclear-Chicago, Type 1620, rate-meter with a beta-sensitive Geiger tube. In 1958 essentially the same procedure was followed, but with only 15,000 tagged larvae from two pools. On this occasion, 10 larvae taken from each of the two tanks the day before their release averaged 7,910 and 8,454 c.p.m. (range 3,000–12,000 and 5,000–13,000) respectively. Temperatures of the test pools were recorded daily from April 25 to May 3, with Taylor maximum–minimum thermometers.

Information on mosquito and predator development was obtained in 1959 from specimens collected from the sample pools at 5-day intervals from April 6 to June 5.

The numbers of potential predators per unit area were estimated by sampling in 1955. This was done with a wooden sampling frame covered on four sides with 24-mesh plastic cloth to enclose an area of 0.5 sq. m. In practice the frame was dropped into the pool and quickly pressed down to prevent the escape of animals trapped on the bottom. The water within the frame was then sieved and the bottom dredged for 10 minutes by two men; captured pond animals 3 mm or more in length were preserved in Freiling's solution. Immature stages were examined and measured in the laboratory with the aid of a dissecting microscope.

Populations of mosquito larvae were sampled with the Belleville trap (12).

## Results and Discussion

### *Evidence of Predation on Radioactive Mosquitoes*

A total of 132 pond animals were obtained from the 1956 experiments and were checked for radioactivity. The radiation counts of 30 of these indicated that they had fed on the tagged *Aedes* larvae (Table I). It was also evident from the counts that some predators had fed more than others on the tagged larvae and were therefore more effective control agents. Three species of Dytiscidae, *Agabus erichsoni* G. & H. (adults and larvae), *Rantus notatus*

TABLE I  
Animals collected in temporary woodland pools, Chatterton, Ontario, in 1956 and 1958, with evidence of feeding on radioactive mosquitoes

Animal	May 1-3, 1956				April 14-16, 1958			
	Total no.	No. radio-active	Average radio-activity, c.p.m.	Range	Total no.	No. radio-active	Average radio-activity, c.p.m.	Range
Gastropoda								
<i>Siagaticola palustris</i>	14	3	1,955	770-3,360	42	19	794	400-1,700
Crustacea								
<i>Crangonyx</i> * sp.	17	1	1,070	—	24	3	1,067	300-2,000
Insecta								
<i>Chastodes</i> sp. (larva)—Sialidae (Neuroptera)	0	0	0	—	1	0	0	—
<i>Gerris</i> ssp.—Gerridae (Hemiptera)	8	3	3,476	168-5,557	0	0	—	—
<i>Gerris comatus</i> * D.&H.—" "	0	0	—	—	12	2	1,750	1,500-2,000
<i>Gerris dissortis</i> " "	0	0	—	—	2	0	0	—
<i>Callicorixa audeni</i> Hungd.—Corixidae " "	8	0	0	—	1	0	0	—
<i>Limnephilus</i> sp. (larva)—Limnephilidae (Trichoptera)	0	0	0	—	77	19	2,932	800-11,000
<i>Acutus senilis</i> Lucas*—Dytiscidae (Coleoptera)	13	12	0,226	1,012-54,571	15	13	8,000	—
<i>A. erichsoni</i> (larva) " "	2	2	3,117	2,725-3,569	1	1	3,760	400-17,000
<i>A. phaeopterus</i> * " "	0	0	—	—	3	1	1,500	—
<i>A. sharpi</i> * " "	0	0	—	—	2	1	2,000	—
<i>Colymbetes sculptilis</i> * " "	0	0	—	—	5	3	1,367	1,000-1,600
<i>Hydroporus fuscicornis</i> * " "	3	1	1,625	—	7	2	900	500-1,300
<i>Hydroporus turbidus</i> * " "	0	0	—	—	2	1	500	—
<i>Hydra notata</i> * " "	1	1	8,551	—	0	0	—	—
<i>Hydra fuscicornis</i> *—Hydrophilidae (Coleoptera)	48	1	871	—	29	0	—	—
<i>Hydrophilus obsoletus</i> " "	16	6	1,164	706-2,697	44	24	2,188	1,300-7,000
<i>Tropisternus mixtus</i> (Lec.) " "	0	0	—	—	1	0	0	—
Arachnida								
<i>Dolomedes</i> sp.* (immature males)	0	0	—	—	12	1	4,000	—

\* Not previously recorded as predators of *A. stimulans* and *A. trichurus*.

(Fab.), and *Hydroporus tenebrosus* Lec., and also the crustacean *Crangonyx* sp., apparently have not been recorded previously as predators of larvae of the two *Aedes* species under study. The remaining predators, with the exception of *Hydrobius fuscipes* (L.), were previously found to feed on late-instar larvae of the same species in permanent pools (2). Twelve specimens, including ephememerid nymphs, hydrachnid mites, and one adult *Hydroporus striola* (Gyll.), were only mildly radioactive above background. These appeared to have been contaminated by ingesting small amounts of radioactive excreta and were thus omitted from the table.

Unseasonably low temperatures during the experiments noticeably reduced the activity of the water beetles. The daily mean temperatures of the test pools from April 30, the day before the tagged larvae were released, until May 3 ranged from 6.7° to 10.0° C. Few specimens were observed swimming and most of them had to be dredged from the bottom and margins of the pools.

In the 1958 experiment, the effect of higher temperatures on predator activity in mid-April was reflected in the collection of 15 additional species, 284 pond animals in all, of which 92 were found to be radioactive, an increase of 9.7% over the number found in 1956. Daily mean pool temperatures for April 13 to April 17 were higher than those of 1956, ranging from 7.8° to 13.3° C.

Seven of the species determined in 1956 and 1958 may be termed resident predators, e.g. *Agabus erichsoni* and *Hydrophilus obtusatus* Say, as they continue to develop annually in the same pools and they are usually abundant. The status of *Colymbetes sculptilis* Harr. is not clear; in some years it is scarce, though there is some evidence that its larvae develop in temporary pools. The remainder, *Agabus sharpi* Fall, *A. phaeopterus* (Kby.), *Hygrotus turbidus* (Lec.), and *Dolomedes* sp., are regarded as occasional predators as they do not always frequent the same pools. In general, on the bases of their radioactivity and numbers, adults of Dytiscidae and of the hydrophilid *H. obtusatus* and the larvae of the caddis-fly *Limnephilus* sp. appeared to be the most effective predators of the early-instar *Aedes*.

#### Other Evidence of Predation

Additional records of predators that had fed on mosquito larvae in temporary pools were obtained by the dissection of five species of water beetles collected on April 12 and 21, 1954. All specimens were examined on the day of collection and the dissected crops were found to contain the remains of *Aedes* larvae, mostly first-instar, as follows:

Date collected	Species	Number dissected	Number with aedine remains	Total number of aedine larvae
April 12	<i>Colymbetes sculptilis</i>	3	3	38
	<i>Gyrinus affinis</i> Aubé	3	3	7
	<i>Agabus</i> sp.	5	1	4
April 21	<i>Acilius semisulcatus</i>	2	2	7
	<i>Gyrinus affinis</i>	8	4	6
	<i>Gyrinus lecontei</i> Fall	8	3	8

It is interesting that water beetles of medium size, such as *Acilius semisulcatus* Aubé and *Colymbetes sculptilis*, should accept first- and second-instar larvae as prey. Both species usually feed on much larger aquatic insects and pond animals in other habitats (13), as well as those in temporary woodland pools in late spring. The capture of such small prey, however, may be due to the limited vision of the Dytiscidae, which in general perceive objects only at close quarters (1), and to the abundance of *Aedes* larvae and various Entomostraca, which together form the most plentiful source of food in early April.

#### *Predators in Relation to Mosquito Development*

Bates (3) indicated that field studies are essential in evaluating the role of mosquito predators, and that "the importance of a given predator would depend upon how far its habits coincided with the habits of mosquitoes". Evidence on this point is scarce, though Curtis (5), Haufe (6), and Sailer and Lienk (11) showed that certain predators are important in the control of northern *Aedes* whose larvae appear either earlier or later in the season than their natural enemies.

Field observations at Chatterton indicated that mosquito development and predator activity are greatly influenced by the weather, particularly temperature, during and following the spring break-up. In some years, high water levels combined with low temperatures delay the growth of the mosquito larvae and at the same time restrict predation to the hardy, early-appearing dytiscids and gyrinid migrants.

In 1956, young larvae of *A. stimulans* were collected at Chatterton on April 6, at the margins of the swamp after 5 days of spring thaw. Adults of two dytiscids, *Colymbetes sculptilis* and *Agabus erichsoni*, were observed on April 12 in pools of water at 4.4° and 7.2° C respectively, though ice to a depth of 15 in. remained in shaded sections nearby. Low temperatures with snow, rain, and much overcast continued to retard mosquito development throughout April with the result that almost all of the larvae of both *Aedes* species stayed for 16 days in the first instar. Predators were not at all conspicuous.

Mosquito development in 1958 commenced 2 weeks earlier than in 1956. First-instar larvae were collected on March 24 and two dytiscids, *Agabus erichsoni* and *Hydroporus* sp., were observed on the next day in water at 7° C. During the field experiment, April 13-17, adult Dytiscidae, Gyrinidae, and Gerridae were active and appeared numerous in the pools.

In 1959, field conditions were recorded from late March until the end of June. The early spring was marked by the rapid thawing of from 2 to 3 ft of snow and ice that had accumulated over unfrozen swamp soil. From March 23 high water extended 15 to 25 yards beyond the usual margins of the swamp for about 1 week and as a result first-instar larvae were not collected until April 4, though some probably hatched several days before this.

Daily mean pool temperatures from April 4 to April 13, when only first-instar *Aedes* were present, ranged from 7.5° to 8.3° C. From April 16 to May 5, an increase in mean temperatures from 10.0° to 15.3° C accelerated late-instar mosquito development resulting in a near-average trend of pupation and emergence in late April and May. Adult predators also were active.

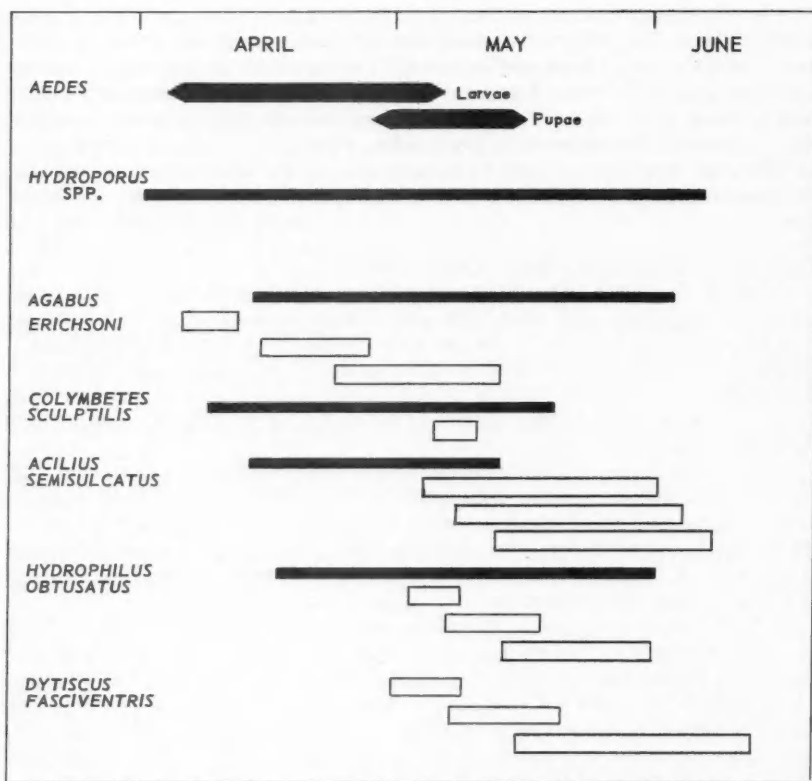


FIG. 1. Occurrence of some predaceous water beetles (narrow black bars) and their larval stages (white bars) in relation to mosquito development in temporary woodland pools, Chatterton, 1959.

The occurrence and development of some of the predaceous water beetles at Chatterton in 1959 are shown in Fig. 1. With one exception, the adult beetles were present throughout the period of mosquito development, though some species were active earlier than others. *Hydroporus* sp., for instance, was observed in the pools at 7° C, before the mosquito larvae had hatched, but 2 days later was seen among populations of newly hatched *Aedes* and *Cyclops*. Other predators, such as *A. erichsoni*, *A. semisulcatus*, *H. obtusatus*, and also *Gyrinus lecontei* Fall (not shown), were not observed until the temperature of the pools had risen to a daily mean of 10° C or more. *Dytiscus fasciventris* Say, a rather large beetle, is usually not encountered until June, long after the mosquitoes have emerged, and apparently enters the pools to prey on the larger pond animals and to oviposit.

Of the water beetle larvae, those of *A. erichsoni* were the most important.

This species was unique in that the occurrence of the larvae is well synchronized with mosquito development. The eggs are deposited in May and June, apparently on moss near the margins of the pools where most of the *Aedes* eggs are laid, and hatch about the same time as *Aedes* in the following March or April. The development of *C. sculptilis* may be similar, though the scarcity of the larvae suggests that this takes place in more permanent water (9). Larvae of the three larger water beetles, *A. semisulcatus*, *H. obtusatus*, and *D. fasciventris*, were not present until early May, when most of the *Aedes* larvae under study had matured. This meant that only the pupae and some retarded larvae were vulnerable to them. Comparable data on the predator fauna of a permanent pool have been reported (2).

#### Predator Numbers

Limited sampling in April, 1955, provided some information on the relative numbers of predators and mosquito larvae (Table II). The samples contained many of the resident predators of the larvae and pupae, and their numbers were representative of samples taken in mid-April.

TABLE II

Predators and mosquito larvae collected in 10 samples from temporary woodland pools at Chatterton, Ontario, on April 18 and 19, 1955

Species	Mean and standard error of animals taken over 0.5 sq. m of pool bottom
<i>Stagnicola palustris</i> (Gastropoda)	0.3 ± 0.2
<i>Limnephilus</i> sp. (larva)—Limnephilidae (Trichoptera)	2.1 ± 0.7
<i>Acilius semisulcatus</i> —Dytiscidae (Coleoptera)	0.1 ± 0.1
<i>Agabus erichsoni</i> " "	0.5 ± 0.2
<i>A. erichsoni</i> (larva) " "	0.6 ± 0.3
<i>A. phaeopterus</i> " "	0.2 ± 0.1
<i>Hydroporus</i> spp. " "	0.6 ± 0.2
<i>Hydrophilus obtusatus</i> —Hydrophilidae "	0.4 ± 0.3
<i>Aedes stimulans</i> —Culicidae (Diptera)	498 ± 171.8
<i>A. trichurus</i> " "	57 ± 21.1

The method of sampling is subject to errors that would tend to underestimate the number of predators. Adult beetles might escape from the sampling frame before the removal of an obstruction at the base; adult dytiscids, and caddisfly larvae, often inactive at low temperatures or immobile from reflex responses when dredged (*A. erichsoni*), could be missed during examination of pond debris.

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The author is indebted to Mr. Derek Riordan for technical assistance in studies with the radioactive tracers. He is grateful also to Dr. A. L. Turnbull for the identification of spiders, and for the identification of aquatic Coleoptera and Hemiptera respectively to Mr. W. J. Brown and Dr. L. A. Kelton, Insect Systematics Unit, Entomology Research Institute, Ottawa.

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## PARASITES FROM NORTHERN CANADA

### II. HAEMATOZOA OF FISHES<sup>1</sup>

MARSHALL LAIRD

#### Abstract

Parasites were present in thin blood films from only 6 of 188 fishes of 11 species, collected in the Northwest Territories and northern Quebec. The haemoflagellate, *Cryptobia gurneyorum* (Minchin), is recorded from North America for the first time—from the type host, *Esox lucius* L., and from two new ones, *Coregonus clupeaformis* (Mitchill) and *Salvelinus namaycush* (Walbaum). *Haemogregarina irkalukpiki* n.sp., characterized by its large size (av., 17.2 by 3.2  $\mu$ ) and nucleophilic habit, is described from two sea-run arctic char, *Salvelinus alpinus* (L.).

#### Introduction

All the blood films discussed herein were collected in the spring and summer of 1959. With the exception of 10 from sticklebacks and 4 from arctic char taken by the author in Ungava, they were obtained by personnel of the Barren Ground Lakes fisheries survey conducted by the Arctic Unit of the Fisheries Research Board of Canada. There have been no previous studies of the haematozoa of fishes from high latitudes in North America.

#### Materials and Methods

Table I lists the fishes examined, the key to collecting stations being as follows:

1. Stream (subject to tidal influence) flowing into False River, Ungava Bay, Quebec (58°07' N, 68°07' W). June.
2. Kasegalik River, Belcher Islands, Hudson Bay, N.W.T. (55°50' N, 79°30' W). August.
3. Maze Lake, N.W.T. (62°25' N, 93°25' W). September.
4. Hyde Lake, N.W.T. (60°45' N, 95°21' W). August.
5. Macdougall Lake, N.W.T. (65°59' N, 98°34' W). August.
6. Angikuni Lake, N.W.T. (62°17' N, 99°36' W). August.
7. Beverly Lake, N.W.T. (64°35' N, 100°38' W). August.
8. Dubawnt Lake, N.W.T. (62°43' N, 102°31' W). August.
9. Ellice Lake, N.W.T. (65°43' N, 106°00' W). August.
10. Lake Beechey, N.W.T. (65°12' N, 106°28' W). August.
11. Whitefish Lake, N.W.T. (62°36' N, 106°43' W). July.
12. Redrock Lake, N.W.T. (65°29' N, 114°24' W). July.
13. Vaillant Lake, N.W.T. (66°12' N, 114°29' W). July.
14. Dismal Lakes, N.W.T. (67°13' N, 116°32' W). July.
15. Lac la Martre, N.W.T. (63°10' N, 117°43' W). June.

A single, thin blood film was made from the heart of each fish. Slides for the purpose and instructions for preparing the films were included in compact

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Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., with financial assistance from the National Research Council of Canada.

TABLE I  
Study material

Systematic position*	Common name*	Collecting stations	No. examined
Clupeiformes			
Salmonidae			
§ <i>Coregonus</i> † <i>artedii</i> LeSueur	Lake herring	2	6
<i>Coregonus clupeaformis</i> (Mitchill)	Lake whitefish	4-7, 13-15	42
§ <i>Prosopium cylindraceum</i> (Pallas)	Round whitefish	5, 7, 11	3
<i>Salvelinus alpinus</i> (L.)	Arctic char	1-3, 5	43
§ <i>Salvelinus namaycush</i> (Walbaum)	Lake trout	3-15	58
§ <i>Thymallus arcticus</i> (Pallas)	Arctic grayling	5, 7, 8	4
Esocidae			
§ <i>Esox lucius</i> L.	Northern pike	4, 7	12
Cypriniformes			
Cyprinidae			
§ <i>Hybopsis</i> ‡ <i>plumbea</i> (Agassiz)	Lake chub	8	1
Catostomidae			
§ <i>Catostomus commersoni</i> (Forster)	Longnose sucker	8, 11	8
Gadiformes			
Gadidae			
§ <i>Lota lota</i> (L.)	Burbot	8	1
Gasterosteiformes			
Gasterosteidae			
<i>Pungitius pungitius</i> (L.)	Ninespine stickleback	1	10

\*As given in the list of common and scientific names of fishes from the United States and Canada, published by the American Fisheries Society (Special Publication No. 2, 1960).

†*Leucichthys* of some authors.

‡*Comesius* of some authors.

§Fresh-water only.

protozoological collecting kits issued to each of the survey parties. The kits will be described in a later paper of this series, dealing with gill parasites. The films were air-dried, fixed in methyl alcohol in the field, and stained with Giemsa's when received at this Institute at the end of the season.

### Descriptive Account

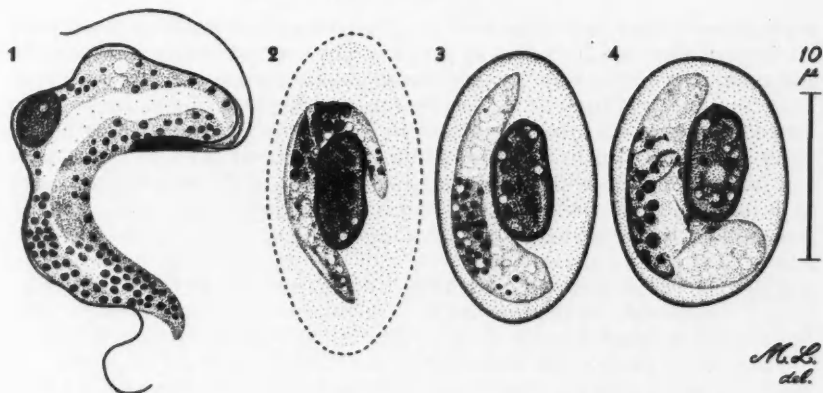
#### MASTIGOPHORA: PROTOMONADINA

*Cryptobia* Leidy (= *Trypanoplasma* Laveran and Mesnil)

*Cryptobia gurneyorum* (Minchin, 1909) (Fig. 1)

*Hosts*: one *Coregonus clupeaformis* (Dismal Lakes), two *Salvelinus namaycush* (Dismal Lakes and Lac la Martre), and one *Esox lucius* (Hyde Lake).

The parasite level was low in each instance, 10 flagellates being the most present in an entire thin film. Air-drying may cause considerable distortion of these organisms, but most of the examples seen are approximately C-shaped as in Fig. 1, a narrow undulating membrane following the convex margin. The nucleus is located near this margin at from one-third to one-half of the body length from the anterior extremity, an elongate kinetoplast lying somewhat in advance of it against the concave margin. Two flagella originate near the anterior end of the kinetoplast, one of them following the outer edge of the undulating membrane to become free a short distance from the posterior extremity. Their separate basal granules are obscured by overdeposition of



FIGS. 1-4. (Figures prepared at a magnification of  $\times 2450$ , with the aid of a Zeiss drawing apparatus.) Fig. 1. *Cryptobia gurneyorum* (Minchin) from *Salvelinus namaycush* (Walbaum), Dismal Lakes. Figs. 3-4. *Haemogregarina irkalukpiki* n.sp. from *Salvelinus alpinus* (L.), Ungava. Erythrocyte shown in Fig. 2 damaged in smearing, the dotted line indicating its approximate contour.

stain about the kinetoplast. While the length of the anterior free flagellum may reach  $19\mu$ , that of the posterior one does not exceed  $10\mu$ . Most individuals have over-all body measurements within the range of  $19.2$  to  $28.2\mu$  by  $3.1$  to  $7.1\mu$  (av. for 17,  $25.1$  by  $6.7\mu$ ). The cytoplasm stains deepest posteriorly and along the convex margin, a central pale zone running longitudinally (Fig. 1). Its denser zones are packed with granules up to  $0.6\mu$  in diameter, which stain dark red. Scattered giant individuals measure up to  $38.8$  by  $9.1\mu$ , but otherwise resemble those already described.

Some authorities (e.g., 8) choose to refer all trypanoplasms (i.e., members of the genus *Cryptobia* from the blood of fishes and salamanders) to a single species, *C. borreli* Laveran and Mesnil (13). There are certainly good grounds for adopting this course in some (10) if not all (24) cases, at least in so far as fresh-water fishes are concerned. Wenyon (24) pointed out that several species have been established following studies of very light infections, warning that it is impossible in such cases to be sure that the forms found do not in fact belong to a single species.

It is submitted that the *Cryptobia* under discussion exhibits constant differences from *C. borreli*. The latter species was first described (13) as measuring about  $20$  by  $3$  to  $4\mu$  in stained dry films, the free flagella each being some  $15\mu$  long. Although Keysselitz (10) illustrated examples with a relatively short posterior flagellum, the sketches published by Laveran and Mesnil (13, 14) clearly indicate that the flagella are coequal. So do those in the plate accompanying Mavor's (16) first record of *C. borreli* from North America, the only previous report of a member of the genus from the blood of a Canadian fish—*Catostomus commersoni* (Lacépède), associated with introduced carp, one of the European hosts for this flagellate, in the Georgian Bay area of Lake Huron. More recently, *C. borreli* has been found in trout

of the genus *Salmo* in California (23). Mavor's over-all body measurements for 50 examples were 20 to 25  $\mu$  by 3 to 4  $\mu$ . The average length of the smaller and commoner form of the present species thus stands at the extreme upper limit for the usual form of *C. borreli*, the average width being appreciably above the upper limit for this flagellate. Nevertheless, giant forms of the latter were described by Keysselitz (10), who declared them to be gametes and integrated them with an imaginative life cycle of the type hypothesized by Schaudinn from confusion of certain quite unrelated blood parasites with one another. Reference to the literature indicates that these flagellates are not only polymorphic, but exhibit different appearances according to the fixation and staining procedures employed. Thus, were the northern *Cryptobia* separated from *C. borreli* only by the features to which attention has just been directed, there would be small grounds for not referring them to the latter species.

They differ, though, not only from *C. borreli* but also from two American species (*C. salmositica* Katz and *C. lynchi* Katz) in which the posterior flagellum is much shorter than the anterior one (9), in having the cytoplasm packed with discrete, chromophilic granules. These are conspicuous in all examples seen, flagellates of average size exhibiting about 100 of them, and they show refraction by phase contrast. They greatly outnumber both the mitochondria of *C. borreli* and the scattered chromatic granules noted in strains of this species from European minnows (14) and Lake Huron *Catostomus* (16). Often masking all other cytoplasmic structures, the granules are certainly the most striking feature of the present species. In possessing them, this species is immediately separable from *C. cyprini* (Plehn), which it otherwise resembles in its over-all size and unequal flagella (20), and from *C. varium* (Léger), with which it is comparable both in size and in exhibiting giant forms (15). These were the second and third species of the genus to be described from the blood of fresh-water fishes. Four further species were proposed by Brumpt (2, 3), one (*C. guernei*) with black pigment granules, another (*C. barbi*) of dimensions similar to the northern Canadian flagellate but lacking granulation, and two (*C. abramidis* and *C. truttae*) that were inadequately described from live individuals alone.

Some at least of the trypanoplasms mentioned in the last paragraph are probably referable to *C. borreli*. None of them is as distinctive as the eighth member of the genus named from a fresh-water fish (English *Esox lucius*), *C. gurneyorum* (Minchin) (18). Its cytoplasm contains very numerous deep-staining granules which, unlike the metachromatic granules of certain fish trypanosomes, hold an iron-haematoxylin stain quite as fast as does the nucleus. In the relative length of the flagella and other morphological respects, as well as in the occurrence of giant forms, there is close agreement between this species and the one under consideration. Minchin (18) failed to provide actual measurements, but Nawrotzky (19) subsequently gave the average dimensions of the common form (from the type host, in Russia) as 18.2 by 7.0  $\mu$ , and those of a giant example as 42 by 11.2  $\mu$ .

It remains to be demonstrated whether or not the euchromatic granules of *C. gurneyorum* consist of ribonucleic acid like those of some trypanosomes (1). If they do, they are not necessarily constant features throughout adult life and their taxonomic value is reduced accordingly. With recognition of

the fact that future histochemical and biological studies may reduce the distinction now arbitrarily drawn between *C. borreli* and *C. gurneyorum*, it is held that the flagellates from northern pike, lake whitefish, and lake trout are properly referable to the latter species.

#### SPOROZOA: COCCIDIA

*Haemogregarina* Danilewsky (sensu lato)

***Haemogregarina irkalukpiki* n.sp.** (Figs. 2-4)

*Host*: two *Salvelinus alpinus* (Ungava and Belcher Islands).

Haemogregarines are rare in both films, all 15 examples seen being intra-erythrocytic. Their length, measured along the mid-line, ranges from 15.7 to 19.4  $\mu$  (av., 17.2  $\mu$ ), and that of the nucleus, from 5.1 to 6.3  $\mu$  (av., 5.7  $\mu$ ). The center of the latter structure is located about 40% of the over-all length from the broader end of the body, the maximum thickness of which varies from 2.5 to 4.3  $\mu$  (av., 3.2  $\mu$ ). Up to 24 chromatin blocks are distinguishable in the nucleus of the parasite, the cytoplasm of smaller (Fig. 2) and average-sized (Fig. 3) individuals of which exhibits two or three subterminal chromatic granules. All but the largest vermicles (Fig. 4) have both extremities bluntly pointed. The cytoplasm is vacuolar, staining pale blue with darker maculations, and altogether lacking the reddish-purple polar caps present in some fish haemogregarines. At all stages the organism is closely applied to one side of the host cell nucleus. One extremity sometimes closely embraces this structure (Fig. 2), which may become slightly displaced (Fig. 4). Although the staining reaction of the cytoplasm of the host cell is not affected, that of its nucleus is, a heavy peripheral deposit of chromatin becoming evident (Figs. 2-4). Invaded erythrocytes show slight if any hypertrophy, the 15 measured ranging from 14.7 to 19.4  $\mu$  (av., 17.0  $\mu$ ) by 9.0 to 11.2  $\mu$  (av., 9.7  $\mu$ ) by comparison with the 14.5 to 20.4  $\mu$  (av., 16.9  $\mu$ ) by 8.4 to 10.6  $\mu$  (av., 9.4  $\mu$ ) for 50, consecutive, normal red cells.

One of the few fish haemogregarines closely comparable in size with the parasite of the arctic char is *H. lignieresi* Laveran, 1906 (12), of Argentinian eels. The host, like the present one, is of partly marine and partly fresh-water habit, and *H. lignieresi* lies in the immediate vicinity of the red cell nucleus and attains dimensions of 20 by 3.5  $\mu$ . However, these large examples lie completely folded upon themselves, their actual length *in situ* being 10 to 11  $\mu$ . *H. bettencourti* França, 1908 (5), of European eels, and *H. thyrsoidae* de Mello and Valles, 1936 (17), of an Indian fresh-water eel, are smaller and not nucleophilic. Only one intraerythrocytic haemogregarine, *H. esocis* Nawrotzky, 1914 (19), of northern pike in Russia, was previously known from any of the fishes dealt with herein. This species, besides showing no attraction to the host cell nucleus, is of a maximum length (11.2  $\mu$ ) well below the minimum figure for the present species. While the latter stands closer to some of the larger haemogregarines of purely marine fishes, it differs from any of those already described in combining unusual size and absence of chromatic polar caps with a highly nucleophilic habit.

Some at least of the species of *Haemogregarina* (sensu lato) parasitizing fishes have intraleucocytic as well as intraerythrocytic stages. This was made

clear when the full blood cycle of *H. bigemina* Laveran and Mesnil was elucidated in New Zealand (11) and subsequently confirmed for a wide range of hosts elsewhere (21, 22). "Leucocytogregarines" noticed in fish from time to time are probably referable to such cycles. This was suspected by Henry (7), who called an organism from the white cells of British haddock (*Haemogregarina aeglefini* Henry not being found in either of the examples concerned) a "leucocytozoon". Henry, it should be noted, employed the term merely because white cells harbored his parasite, which he did not regard as congeneric with *Leucocytozoon* Danilewsky. Fantham, Porter, and Richardson (4) certainly erred in assigning their *L. salvelini*, described from the polymorphonuclear and mononuclear leucocytes of Quebec brook trout, to this genus. Only three of several hundred examples of *Salvelinus fontinalis* (Mitchill) harbored *L. salvelini*, which, from the illustrations accompanying its description, is obviously a blood coccidian. The maximum measurements recorded for its vermicles, 18.9 by 3.7  $\mu$ , are so close to those of the arctic char haemogregarine as to suggest that the two may prove identical. However, Fantham and his collaborators found no intraerythrocytic stages, while no intraleucocytic ones accompanied the rare haemogregarines described herein. Should further studies link these parasites in a single cycle, the name *Haemogregarina salvelini* (Fantham *et al.*, 1942) would take priority under Article 27 of the International Rules of Zoological Nomenclature. Pending such an association, though, the present species must be held distinct; especially as it was only found in sea-run arctic char, for there is nothing in the account by Fantham *et al.* to suggest that they were dealing with any other than landlocked brook trout.

Sea-run arctic char are called *irkalukpik* by Canadian Eskimos of the eastern Arctic, the haemogregarine described herein accordingly being designated *Haemogregarina irkalukpiki* n.sp. The type slide has been deposited in the collection of the U.S. National Museum, Washington, D.C. (catalogue number 23676).

### Discussion

Species of *Cryptobia* are seldom plentiful in blood films. They are thus easily overlooked, although European investigators have shown that they parasitize a wide range of fresh-water fishes. Some of the known European hosts (e.g., *Esox lucius*, *Lota lota*) and various other fishes having a circumpolar distribution (25), it is not remarkable that these haemoflagellates should now be reported from Canadian Arctic and Subarctic regions. It is more surprising that there is but one previous record (16) of the genus from Canada. Although much more attention has been paid to helminthic than to protozoan parasites of fresh-water fishes in this country, many such fishes, including several hundred brook trout, were examined for haematozoa during extensive surveys in the eastern Provinces rather more than 20 years ago (4). Aside from "*Leucocytozoon salvelini*", the positive findings comprised one infection with *Dactylosoma salvelini* Fantham *et al.*, 1942, and one (in a yellow perch) with *Trypanosoma percae canadensis* Fantham *et al.*, 1942.

More intensive northern studies might result in further records for these and other haematozoa of similarly low incidence in nature. In conclusion,

attention is again drawn to the occurrence of *Haemogregarina esocis* (19), one of the few well-described members of its genus affecting purely fresh-water fishes, in *Esox lucius* in Russia. While most other species reported from such hosts in Europe (e.g., 6) are of uncertain status (24), it might repay Russian investigators to study the possible relationship between *H. esocis* and an inadequately characterized intraleucocytic haemogregarine, *Hepatozoon* (= *Leucocytozoon*) *ninae kohl-yakimovi* Yakimoff, 1917, found in an undetermined fish from a Transcaucasian river (26).

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## SOMATIC CHROMOSOMES OF HIGHER DIPTERA

### V. INTERSPECIFIC AND INTRASPECIFIC VARIATION IN THE CALLIPHORIDAE<sup>1</sup>

J. W. BOYES

#### Abstract

Somatic complements of 12 chromosomes have now been reported for 20 species of Calliphoridae, including *Callitroga macellaria*, *Protocalliphora aenea*, *P. avium*, *P. hirundo*, *P. metallica*, *P. n. sp. near sialia*, *P. sialia*, *Phaenicia eximia*, *Eucalliphora lilaea*, *Cynomyopsis cadaverina*, and *Pollenia rudis*, added in this report. Idiograms are presented of the karyotypes of most of these 11 species and several others concerning which only limited data were previously available. The sex chromosomes of certain species appear to have features common to the genera, tribes, and superfamilies to which they belong, but these features, and others involving autosomal pairs, cannot be considered as characteristic of the larger groups until data are available for the chromosomes of more of the species included in them. Data presented for different collections of six species suggest that some species have progressed further than others in the accumulation of intraspecific chromosomal differences of the kinds that are characteristic of interspecific chromosomal variations in higher Diptera.

#### Introduction

The Calliphoridae (blowflies) are of common occurrence and world-wide in their distribution but little is known about their chromosomes. During the last 8 years I have accumulated some information on this subject and the present report describes in detail the karyotypes of some species, compares the karyotypes of different collections of six, and provides idiograms of a dozen species. Thus, including observations reported herein, information is now available regarding the karyotypes of 20 species of this family.

The available earlier literature on chromosomes of calliphorids was reviewed in the first paper of this series (5); however, it should be mentioned that *Calliphora erythrocephala* Meigen of Metz (11), Keuneke (10), etc. is now replaced by *Calliphora vicina* R.-D., and accordingly a collection received from Weybridge, England, as *C. erythrocephala* is considered to be *C. vicina*. Strasburger (14) has illustrated the 12-chromosome complements of *C. erythrocephala* in both somatic and meiotic divisions but his drawings are not suitable for measurement. *Lucilia sericata* Meig. has been considered to be *Phaenicia sericata* (Mg.) and a collection from Weybridge labelled *Lucilia sericata* is referred to as *P. sericata*. In the recent literature, Fish (6) has described mitotic divisions in embryonic tissue of *Lucilia sericata*, in a thesis which I have not seen, and Kaufman and Wasserman (9) have illustrated the 12-chromosome karyotype of *Callitroga hominivorax* (Coq.) and prepared an idiogram of the species.

An effort has been made to obtain more detailed data from the drawings provided by various authors in the literature. The results of these efforts are

<sup>1</sup>Manuscript received April 4, 1961.

Contribution from the Department of Genetics, McGill University, Montreal, Que.

TABLE I  
Analyses of karyotypes of species of Calliphoridae in published illustrations

Species	Author and reference	Figure no. analyzed	Tissue	Y	Chromosome pair					
					I	II	III	IV	V	VI
Arm ratios		2		—	4.50*	5.15	1.07	1.63	1.93	1.72
1. <i>Callitroga hominivorax</i> (Coq.)	Kaufman and Wasserman (9)	53, 54, 56	(Idiogram)	—	—	—	Med.	—	Med.	Low
2. <i>Calliphora erythrocephala</i>	Metz (10)	1, 36	Ovarian Spermatogonial	—	Acro.	High	2.30	1.12	1.15	1.24
3. <i>Calliphora erythrocephala</i>	Keunike (10)	143	Oogonial, spermatogonial	—	Med.	Med.	High	Med.	Med.	Low
4. <i>Calliphora erythrocephala</i>	Naville (12)	73, 74	Spermatogonial	—	Acro.	1.33	—	—	—	1.22
5. <i>Lucilia caesar</i>	Keunike (10)	38, 39	Oogonial	—	—	—	—	—	—	1.32
6. <i>Phormia regina</i>	Metz (11)			—	1.00*	High	High	High	Low	Low
7. <i>Phormia terrae-novae</i>	Naville (12)			—	—	—	—	—	—	—
Percentages of TCL†				3.5	12.4	13.7	16.1	16.7	18.6	22.5
1. <i>C. hominivorax</i>				—	5.1	16.6	17.4	19.0	20.4	21.4
2. <i>C. erythrocephala</i>				5.1	8.8	13.9	16.8	16.8	21.0	22.8
3. <i>C. erythrocephala</i>				3.1	5.7	16.3	18.2	18.6	18.9	22.3
4. <i>C. erythrocephala</i>				7.5	9.5	15.4	16.4	16.9	20.9	20.9
5. <i>L. caesar</i>				3.1	8.3	16.0	18.3	18.5	19.4	19.5
6. <i>P. regina</i>				—	7.3	16.4	17.8	19.0	19.1	20.4
7. <i>P. terrae-novae</i>				—	—	—	—	—	—	—

\*Secondary ratios in acrocentric chromosomes.

†TCL = total complement length using 2X and omitting the Y-chromosome.

recorded in Table I. The considerable possibility of error in such analyses will be obvious. These results will be considered, in relation to the results of this study, in the discussion.

### Materials and Methods

For all species except *Pollenia rudis* the chromosome complements analyzed were from larval "brain" cells. Some of the larvae were from stock cultures obtained from Weybridge, England, in 1953 through the courtesy of Mr. Tom Spence. Other cultures were contributed as identified larvae by Dr. Gordon Bennett of the Ontario Research Foundation in 1956. A number of collections were reared from adult females captured during a trip to the Caribbean in 1957 and some were reared from adult females captured in or near Montreal. Adults of *Pollenia rudis* were collected at Macdonald College by Dr. W. F. Grant. Other details of the sources of collections and their identification have been recorded in Table II, and in the acknowledgments.

The cytological techniques used in this study were very similar to those described previously (1, 2, 5). The larval "brains" received from Dr. James (see Table II) were fixed by him and Dr. Kamal in Newcomer's fixative. It was found necessary to soak them for about 5 hours in water before staining and

TABLE II  
Sources and identifications of species collections

Species collection	Where obtained	By whom identified
<i>Callitroga hominivorax</i> (Coquerel)	Univ. of Texas, U.S.A., 1957	Stock culture, Kaufman and Wasserman
<i>Callitroga macellaria</i> (Fab.)	St. Georges, Grenada, 1957	G. E. Shewell
<i>Phormia regina</i> (Mg.)		
Collection 1	Algonquin Park, Ont., 1959	C. Sabrosky
2 and 3	St. Hilaire, Que., 1959	G. E. Shewell
<i>Protophormia terraenovae</i> R.-D.	Weybridge, England, 1953	Stock cultures, Spence
	Pullman, Wash., U.S.A., 1954	Stock culture, James
<i>Protophormia</i> species	Algonquin Park, Ont., 1956	G. F. Bennett
<i>Lucilia illustris</i> (Mg.)		
Collection 1	Algonquin Park, Ont., 1959	C. Sabrosky
2	Algonquin Park, Ont., 1959	G. E. Shewell
3	Montreal, Que., 1959	G. E. Shewell
4	Weybridge, England, 1953	Stock cultures, Spence
<i>Phaenicia eximia</i> (Wd.)	Port of Spain, Trinidad, 1957	G. E. Shewell
<i>Phaenicia sericata</i> (Mg.)		
Collection 1	Montreal, Que., 1959	G. E. Shewell
2	St. Hilaire, Que., 1959	G. E. Shewell
3	Weybridge, England, 1953	Stock cultures, Spence
<i>Eucalliphora lilaea</i> (Walk.)	Pullman, Wash., U.S.A., 1955	Stock culture, James
<i>Calliphora vicina</i> R.-D.		
Collection 1, 2, and 3	Montreal, Que., 1959	G. E. Shewell
4	Weybridge, England, 1953	Stock cultures, Spence
<i>Calliphora vomitoria</i> (L.)		
Collection 1	Algonquin Park, Ont., 1959	C. Sabrosky
2	Weybridge, England, 1953	Stock cultures, Spence
<i>Cynomyopsis cadaverina</i> (R.-D.)		
Collection 1	Algonquin Park, Ont., 1959	C. Sabrosky
2	Montreal, Que., 1959	G. E. Shewell
3	Pullman, Wash., U.S.A., 1955	Stock culture, James
4	Weybridge, England, 1953	Stock cultures, Spence
<i>Pollenia rudis</i> (Fab.)	Macdonald College, Que., 1960	G. E. Shewell

TABLE III  
Somatic chromosome complements of species in the subfamily Chrysomyinae

Collection	← Secondary ratios →				← Primary arm ratios →				V	VI	Averages II-VI	No. comp. analyzed	
	Y	I	II	←	II	III	IV	→					
<i>Callitroga hominivorax</i>	—	—	—	—	1.38 ± 0.214	3.11 ± 0.752	1.38 ± 0.187	1.38 ± 0.187	1.58 ± 0.279	1.43 ± 0.228	1.78 ± 0.197	9	
<i>Callitroga macellaria</i>	—	—	—	—	1.28 ± 0.170	1.46 ± 0.241	2.18 ± 0.396	1.48 ± 0.155	1.48 ± 0.155	1.30 ± 0.179	1.54 ± 0.114	18	
<i>Phormia regina</i>	1.25 ± 0.354	0.331 <sup>a</sup>	1.83 <sup>a</sup>	3.50 <sup>a</sup>	1.29 ± 0.130	1.32 ± 0.148	1.95 ± 0.173	1.39 ± 0.187	1.39 ± 0.187	1.38 ± 0.141	1.46 ± 0.063	11	
1. Alenquin Park	0.67 <sup>a</sup>	—	0.321 <sup>a</sup>	1.72 <sup>a</sup>	1.24 ± 0.158	1.38 ± 0.164	1.95 ± 0.263	1.33 ± 0.182	1.33 ± 0.182	1.32 ± 0.126	1.45 ± 0.089	10	
2. St. Hilaire	1.50 <sup>a</sup>	—	0.23 <sup>a</sup>	1.63 <sup>a</sup>	1.27 ± 0.122	1.28 ± 0.134	1.93 ± 0.110	1.31 ± 0.164	1.31 ± 0.164	1.30 ± 0.130	1.42 ± 0.045	12	
3. St. Hilaire	0.80 <sup>a</sup>	—	0.54 <sup>a</sup>	1.74 <sup>a</sup>	1.19	—	1.78	1.22	—	1.34	—	1.45	2
<i>Protophormia terraenovae</i>	0.54 ± 0.210	0.23 <sup>a</sup>	1.52 <sup>a</sup>	2.08 ± 0.175	1.56 ± 0.142	1.29 ± 0.151	1.24 ± 0.149	1.19 ± 0.092	1.19 ± 0.092	1.48 ± 0.059	—	11	
<i>Protophormia aenea</i>	1.08 ± 0.113	0.26 <sup>a</sup>	0.81 <sup>a</sup>	2.05 <sup>a</sup>	2.59 ± 0.250	1.46 ± 0.135	1.58 ± 0.195	1.41 ± 0.133	1.41 ± 0.133	1.23 ± 0.087	1.65 ± 0.063	10	
<i>Protophormia autumnalis</i>	1.23 ± 0.040	0.15 <sup>a</sup>	0.70 <sup>a</sup>	2.89 <sup>a</sup>	2.02 ± 0.133	1.58 ± 0.079	1.49 ± 0.114	1.26 ± 0.142	1.26 ± 0.142	1.26 ± 0.093	1.52 ± 0.045	10	
<i>Protophormia metallica</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
Percentages of TCL													
<i>Callitroga hominivorax</i>	4.70 ± 0.424	8.83 ± 1.028	—	—	16.53 ± 0.510	16.93 ± 0.917	17.52 ± 0.686	19.00 ± 0.964	19.00 ± 0.964	21.18 ± 0.987	38.9 ± 5.937	9	
<i>Callitroga macellaria</i>	3.35 ± 0.459	4.54 ± 0.900	—	—	16.26 ± 1.176	18.13 ± 0.830	18.84 ± 1.005	19.79 ± 0.960	19.79 ± 0.960	22.48 ± 1.353	41.6 ± 8.350	18	
<i>Phormia regina</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
1. Alenquin Park	3.94 ± 0.993	8.3 ± 0.784	—	—	16.8 ± 0.622	17.5 ± 0.369	18.0 ± 0.560	18.2 ± 0.706	18.2 ± 0.706	21.2 ± 1.131	64.7 ± 7.051	11	
2. St. Hilaire	4.2 ± 0.893	9.8 ± 1.306	—	—	16.0 ± 0.672	17.4 ± 0.685	17.7 ± 1.142	18.3 ± 0.991	18.3 ± 0.991	20.8 ± 1.066	56.5 ± 8.396	10	
3. St. Hilaire	5.11	9.8 ± 0.920	—	—	15.9 ± 0.523	17.3 ± 0.626	17.7 ± 0.880	18.7 ± 0.759	18.7 ± 0.759	20.6 ± 0.986	57.8 ± 6.629	12	
<i>Protophormia terraenovae</i>	3.31	7.0	—	—	16.6	—	18.6	19.1	—	22.0	—	76.53	2
<i>Protophormia aenea</i>	4.0 ± 0.696	7.4 ± 0.595	—	—	16.3 ± 0.499	17.4 ± 0.758	17.7 ± 0.784	18.7 ± 0.791	18.7 ± 0.791	22.6 ± 1.535	63.57 ± 15.258	12	
<i>Protophormia autumnalis</i>	5.4 <sup>a</sup>	6.8 ± 0.854	—	—	16.0 ± 0.523	16.9 ± 0.211	17.7 ± 0.558	19.2 ± 0.391	19.2 ± 0.391	23.3 ± 1.132	68.8 ± 4.939	10	
<i>Protophormia metallica</i>	5.2 ± 0.804	10.0 ± 0.359	—	—	15.6 ± 0.823	16.4 ± 0.528	17.4 ± 0.379	18.5 ± 0.357	18.5 ± 0.357	22.1 ± 0.892	75.5 ± 10.651	10	

NOTE: Superscript figures indicate the number of individual chromosomes involved.

to reduce the staining time to about 17 minutes. The effect of such treatment on the chromosomes is not known but in the case of *Cynomyopsis cadaverina* the results obtained from chromosome analysis are quite comparable with those obtained using living larvae.

The methods of analysis have also been described previously (1, 5). In some preparations secondary constrictions are conspicuous and of value in identifying members of particular pairs of chromosomes. In recording the positions of these constrictions in chromosome arms the writer has consistently divided the length of the segment closer to the kinetochore (i.e. the proximal segment) by the length of the segment further away from the kinetochore (i.e. the distal segment) to obtain a statistic which is referred to as a secondary arm ratio. Sometimes two secondary ratios have been determined for the same chromosome arm. The long arm of each pair is referred to as L and the short arm as S.

Photomicrographs of the karyotypes of several species are shown in Figs. 13-17. Many of these karyotypes are so similar that differences are only obvious in the data, and accordingly illustration of them, except for the idiograms, has been omitted. The terms heterochromatic and heteropycnotic are used as defined previously (5).

### Descriptions of Somatic Metaphase Complements

#### SUBFAMILY CHRYSOMYINAE

##### TRIBE CHRYSOMYINI

#### *Callitroga hominivorax* (Coquerel)

Larvae of this species, known as the screwworm, were obtained from Drs. Kaufman and Wasserman, who described briefly the somatic mitotic chromosomes from brain and spermatogonial cells including an idiogram of the chromosome complement (9). My observations, based on analyses of nine chromosome complements of brain cells from three larvae (presumed to be 2 ♀, 1 ♂), agree in general with theirs but differ in certain details. The complements analyzed ranged in TCL from 31.5 to 42.7  $\mu$  with 38.9  $\mu$  being the average (see Table III and Fig. 1).

The acrocentric pair I averaged just under 9% and a secondary constriction separates proximal:distal segments in the ratio of about 4:1. The small acrocentric Y-chromosome corresponds to about 4.7% of the TCL and is easy to recognize. Pair II (probably corresponding to Kaufman and Wasserman's No. 3) is much longer and differs strongly from pair III in arm ratio. The high arm ratio, 3.11, for pair III helps also in distinguishing chromosomes of this pair from those of pairs IV and V. Pair V is slightly longer than IV and has a slightly higher arm ratio, so that these pairs are moderately distinct. Pairs IV and V are distinctly shorter than pair VI.

Kaufman and Wasserman (9) do not show in their idiogram any secondary constrictions in the autosomes of this species and few were seen by me. There are, however, distinct constrictions dividing the long arm of pair III with a secondary ratio of 0.61 for the nine chromosomes in which they were seen, and occasionally others, as shown in Table VII. The shortness of the complements analyzed may account for the scarcity of secondary constrictions seen, since such constrictions are often difficult to identify in short, compact chromosomes.

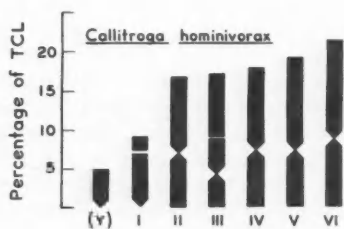


Figure 1

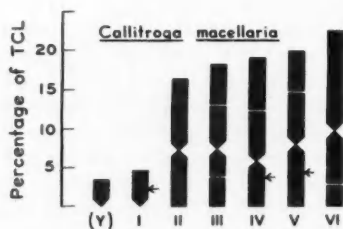


Figure 2

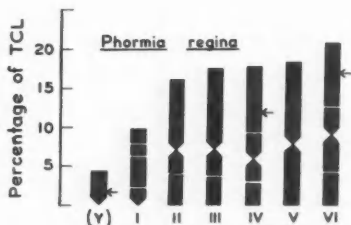


Figure 3

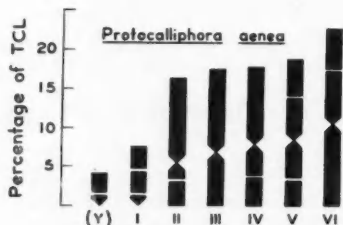


Figure 4

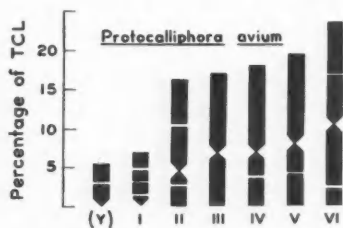


Figure 5

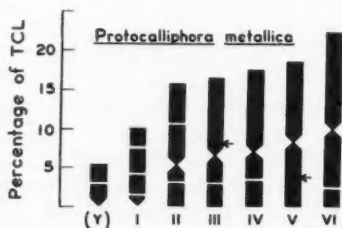


Figure 6

FIGS. 1-6. Idiograms of somatic chromosomes of species of Calliphoridae. The frequently observed secondary constrictions are represented by a white line across the chromosome in the idiogram, the thickness of the line increasing with the frequency of observation. An arrow at the side of a chromosome indicates that a secondary constriction is sometimes seen at that position and a broken line at the side indicates that a secondary constriction is occasionally found in that position.

*Callitroga macellaria* (Fab.)

Eighteen somatic complements, from six presumed male and one presumed female larvae of this species, known as the lesser screwworm, were analyzed. The larvae were from two different collections taken in Grenada but the results of analyses from the two collections were so similar that it was decided to combine them. The complements ranged from 21.3 to 52.4  $\mu$  in TCL, with an average of 41.6  $\mu$  (see Table III and Fig. 2).

The acrocentric pair I is much smaller in this species than in *C. hominivorax* and constitutes only 4.5% of the TCL. The acrocentric Y-chromosome is even smaller, corresponding to 3.3% of the TCL. Pair II is considerably longer, but usually distinctly shorter than pair III and has a lower arm ratio. Pair IV is only slightly longer than pair III on the average, but its high arm ratio clearly distinguishes it from pairs III and V. Pair VI is noticeably longer than V and also has a somewhat lower arm ratio.

Secondary constrictions were found in many chromosomes (Table VII). It is probable that the secondary constrictions could be found more regularly in longer complements, or with better staining. In the cells analyzed, however, they are not of great value in distinguishing the chromosome pairs.

## TRIBE PHORMIINI

*Phormia regina* (Mg.)

This species is widely distributed and fairly common. As already mentioned, the karyotype of this species has been illustrated previously (11), but not by an idiogram. Thirty-three complements from 7 presumed male and 11 presumed female larvae, taken from three different collections, have been analyzed. The average TCL for each collection is shown in Table III and the over-all range is from 45.4 to 77.5  $\mu$ . An idiogram for the species, based on analysis of complements from collection No. 2 of Saint Hilaire, is shown as Fig. 3.

The acrocentric and heteropycnotic pair I ranged from about 8% to 10% of the TCL and has three frequently observed secondary constrictions providing proximal:distal ratios as recorded in Table III. The acrocentric Y-chromosome is very small, corresponding to only 4.2% of the TCL. Parts of the X- and Y-chromosomes stain lighter than other parts at metaphase. Pair II is longer than pair I, slightly shorter than pair III, and has a slightly lower arm ratio. Pair IV has a distinctive, high arm ratio which is of value in separating it from pairs III and V, a distinction greatly aided by an obvious secondary constriction in its short arm. The arm ratios of pairs V and VI are quite similar but pair VI is decidedly longer. These features together with the secondary constrictions make it possible to identify the members of each pair with considerable confidence.

Secondary constrictions of pair I have been mentioned. A secondary constriction was also found in several Y-chromosomes. Secondary ratios of autosomes are recorded in Table VII. The more common and conspicuous of the secondary constrictions in the autosomal chromosomes are clearly in the short arms of pairs II, IV, and VI.

The three collections of this species yielded very similar results. Primary arm ratios of autosomes are very similar and the slightly lower value of one

secondary ratio in Pair I of collection No. 3 is probably not important. Since heterochromatic sex chromosomes tend to constitute a smaller percentage of the TCL in longer complements (3, 4), the slightly lower percentage for pair I and the Y in the Algonquin Park collection is understandable. This low value also explains the slightly higher percentages of TCL for most of the autosomes of that collection. Secondary ratios in the arms of the autosomes of the three collections were very similar: in IIIS 0.96 for 5, 0.94 for 6, and 0.96 for 8; in IVS 1.05 for 19, 1.06 for 11, and 1.06 for 21; in VIS 1.16 for 4, 1.32 for 4, and 1.18 for 15; and in VII they are 0.43 for 4, 2.24 for 4, and 0.57 for 8. Although St. Hilaire and Algonquin Park are about 400 miles apart the karyotypes of collections from these two places are similar.

The presence of small dense masses in the peripheral parts of metabolic nuclei and other observations indicate that the sex chromosomes are heteropycnotic and heterochromatic. Tetraploid metaphases were occasionally seen.

*Protophormia terraenovae* R.-D.

The 12-chromosome karyotype of this species has been illustrated by Naville (12) as that of *Phormia terrae-novae*. Results of my analyses of one complement from a larva of each of two separate collections have been summarized in Table III. One complement was 63.0 and the other 85.0  $\mu$  in TCL. A partial analysis of the chromosomes in one of Naville's illustrations has been given for comparison in Table I. It seems best to delay preparing an idiogram until more data are available.

The data in Table III indicate that pair I averages about 7% and that the Y corresponds to about half of that value. Pair II has a low arm ratio in the complements drawn by me but Naville's illustration suggests it may be high. Pairs III and IV have arm ratios of about 1.75 and pairs V and VI of about 1.2 and 1.3. Pair VI appears to be considerably longer than pair V.

*Protocalliphora aenea* S. and D.

Twelve complements from three presumed male and three presumed female larvae were analyzed. The complements ranged from 35.6 to 82.1  $\mu$  in TCL, with 63.5  $\mu$  being the average. Details of the analysis have been recorded in Table III and the idiogram is shown in Fig. 4.

The acrocentric pair I constitutes 7.4% of the TCL. Pair II is much longer and has a high arm ratio, averaging 1.56. Pair III is longer than II with an arm ratio of 1.29 and very similar to that of pair V. Pairs IV and V differ little in length. Pair VI also has a similar arm ratio but its greater length distinguishes it from pair V.

Secondary constrictions divide pair I chromosomes into three segments producing secondary arm ratios of 0.23 for five chromosomes and 1.52 for four. The small Y-chromosome has a secondary constriction at a position corresponding to the more proximal one on the X and it gives a secondary ratio of 0.54. As seen in Table VII, secondary constrictions were not often recorded for most autosomal pairs. The most useful ones for distinguishing pairs of chromosomes are those in the short arms of pairs II, IV, and V.

*Protocalliphora avium* S. and D.

Ten 12-chromosome complements from four presumed female and one

presumed male larvae were analyzed. The complements ranged from 58.5 to 76.7  $\mu$ , and averaged 68.8  $\mu$  in TCL (see Table III and Fig. 5).

Chromosomes of the acrocentric pair I are divided by secondary constrictions and they constitute 6.8% of the TCL. The Y-chromosome is also acrocentric and corresponds to 5.4% of the TCL. Pair II is considerably larger and has a high arm ratio, averaging 2.59. Pairs III and IV do not differ greatly in length and have similar arm ratios. Pair V is noticeably longer than IV and shorter than VI but its arm ratio resembles that of IV and is appreciably higher than that of VI. Pairs III and IV are the most difficult to separate on these bases.

In pair I, segments gave secondary ratios of 0.26 for six, 0.81 for four, and 2.05 for five. The Y-chromosomes are divided into two nearly equal segments by secondary constrictions. Using ratios shown in Table VII, with the percentages and arm ratios, the pairs can be identified fairly accurately.

*Protocalliphora metallica* (Tns.)

Ten 12-chromosome complements from three presumed male and two presumed female larvae were analyzed. The complements ranged from 56.9 to 90.4  $\mu$  and averaged 75.5  $\mu$  in TCL (see Table III and Fig. 6).

The acrocentric chromosomes of pair I constitute about 10% of the TCL. The Y is also acrocentric and corresponds to 5.2% of the TCL. Pair II is considerably longer than I and its arm ratio, averaging 2.02, is appreciably higher than that of pair III, which averages 1.59. Pair IV is somewhat longer than III but differs only slightly from it in arm ratio. Pair V is a little longer than pair IV and usually noticeably lower in arm ratio. It is much shorter than pair VI, which it resembles in arm ratio.

Secondary constrictions divide pair I chromosomes into four segments, which give 0.15 for 9, 0.70 for 15, and 2.89 for 9, as secondary ratios of the segments. The Y has 1.23 for four as a corresponding ratio. It will be apparent that the secondary constrictions (see Table VII) are not particularly useful in separating chromosomes of pairs III and IV and hence some confusion between these pairs may complicate analysis.

*Protocalliphora hirundo* S. and D.

*Protocalliphora* n.sp. near *sialia*

*Protocalliphora sialia* S. and D.

All three species have 12-chromosome somatic complements and their chromosomes are similar to those of the other species of *Protocalliphora* mentioned previously. The karyotype of *P. hirundo* is shown in Fig. 15. Details regarding these species will be published in a separate report on the genus *Protocalliphora*.

SUBFAMILY CALLIPHORINAE

TRIBE LUCILIINI

*Lucilia caesar* L.

In 1908 Stevens (13) illustrated the six pairs of chromosomes of this species, including a very large "heterochromosome" bivalent, in a spermatocyte. Later Keuneke (10) described six pairs of somatic chromosomes, including a small heteromorphic pair and five pairs that appear to be metacentric. My analysis from his illustrations is shown in Table I. It is obvious that these two

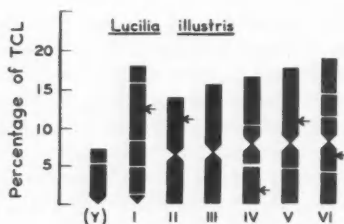


Figure 7

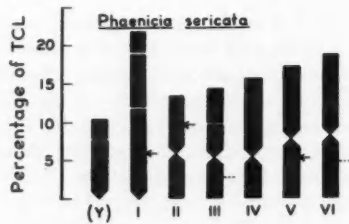


Figure 8

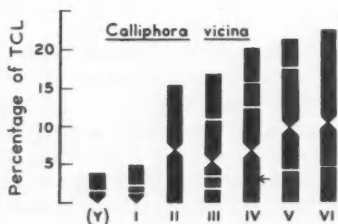


Figure 9

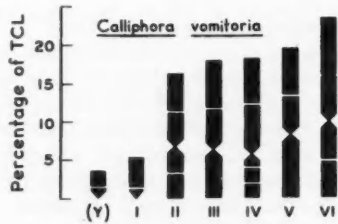


Figure 10

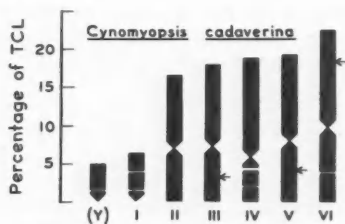


Figure 11

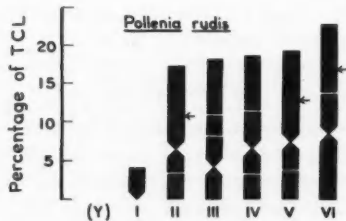


Figure 12

FIGS. 7-12. Idiograms of somatic chromosomes of species of Calliphoridae.

authors do not agree regarding the heteromorphic pair. The following quotation from Hall (8) sheds some light on this problem: "The *Lucilia caesar* of North American authors is usually *Lucilia illustris* (Meigen). Coquillett determined a number of species of both *Phaenicia* and *Lucilia* as *caesar*, as did other North American dipterists early in the present century, and many of the earlier references to either *caesar* or to *illustris* may be inaccurate." From my observations it also seems likely that Stevens may have had *L. illustris* but it is interest-

ing that *L. caesar*, which seems closely related to *L. illustris*, should have a small heteromorphic pair as described by Keuneke.

*Lucilia illustris* (Mg.)

Complements for analysis came from five larvae (presumed to be 1 ♂, 4 ♀) of collection No. 1 (see Table II), two larvae (presumed to be 1 ♂, 1 ♀) of collection No. 2, three presumed female larvae of collection No. 3, and four larvae (presumed to be 2 ♂ and 2 ♀) of collection No. 4. The average length of the TCL for each of the four collections is shown in Table IV. The ranges in TCL are, in the order listed, 64.5 to 83.4, 61.1 to 81.0, 55.1 to 59.1, and 47.2 to 68.0  $\mu$ . Details of analyses have been recorded in Table IV; an idiogram for the species, constructed on the basis of data from collection No. 2, is shown in Fig. 7; and Figs. 13 and 14 are photomicrographs of the karyotype.

Chromosomes of pair I are acrocentric, heteropycnotic, heterochromatic, and ranged from about 16 to 19% of the TCL. They are not difficult to distinguish from chromosomes of pairs V and VI which they resemble in length. The Y-chromosomes are also acrocentric, heteropycnotic, and heterochromatic and correspond to about 6 to 9% of the TCL. Pair II is easy to identify as the shortest with a nearly median kinetochore. Pair III is longer than II and has a higher arm ratio than either II or IV. Pairs IV and V are not easy to distinguish but pair VI is noticeably longer.

Secondary constrictions are common in pair I chromosomes and provide the secondary ratios as shown in Table IV, and in the frequencies shown in the superscript numbers in that table. The Y-chromosome has also a secondary constriction giving a proximal:distal ratio of 2.63 for three. From the secondary ratios given in Table VII for a total of 46 chromosomes of each pair it will be apparent that only those in I, IVS, IVL, VS, VIS, and VIL were seen often enough to be of value in assigning chromosomes to their respective pairs.

The results for the different collections are fairly similar. Bearing in mind that the heteropycnotic pair I chromosomes tend to be a smaller percentage as the TCL increases (3, 4), the percentages of TCL are remarkably similar for each of the pairs in collections 1 to 3, but pair I is noticeably smaller in collection 4 from Weybridge. The average secondary arm ratios of pair I for the latter collection also do not agree well with those of other collections. Other differences, such as the higher arm ratio for pair V in collection No. 3, seem less important. Secondary constrictions in the autosomal pairs show much greater differences in location in the different collections than do those of *P. regina*. It seems possible that the Weybridge collection comes from a different race and perhaps from a distinct sibling species.

*Phaenicia eximia* (Wd.)

A single 12-chromosome complement was obtained in a presumed female larva reared from a fly captured near Port of Spain, Trinidad. The results of analysis of this complement have been recorded in Table IV. No secondary constrictions were recorded. Since both the original female and three progeny reared from it were all identified as this species, the identity of the larva used seems fairly definite, but the chromosomes differ greatly from those of both *Lucilia illustris* and *Phaenicia sericata*.

TABLE IV  
Somatic chromosome complements of species in the subfamily Calliphorinae, tribe Lucilini

Collection	Secondary ratios				Primary arm ratios						Averages II-VI	No. comp. ana- lyzed	
	Y	I	II	III	IV	V	VI						
Arm ratios													
<i>Lucilia illuistris</i>													
1. Algonquin Park	0.14 <sup>1</sup>	0.92 <sup>1</sup>	0.10 <sup>1</sup> , 0.92 <sup>1</sup> , 9.59 <sup>1</sup>	1.28 ± 0.134	1.21 ± 0.141	1.19 ± 0.022	1.21 ± 0.152	1.34 ± 0.158	1.24 ± 0.063	6			
2. Algonquin Park	2.63 <sup>1</sup> ± 0.322	0.07 <sup>1</sup> , 0.38 <sup>1</sup> , 0.86 <sup>1</sup> , 7.82 <sup>1</sup>		1.18 ± 0.089	1.35 ± 0.105	1.12 ± 0.110	1.23 ± 0.130	1.25 ± 0.179	1.22 ± 0.032	8			
3. Montreal	—	—	0.08 <sup>1</sup> , 0.45 <sup>1</sup> , 2.19 <sup>1</sup> , 7.90 <sup>1</sup>	1.11 ± 0.032	1.41 ± 0.212	1.15 ± 0.145	1.44 ± 0.161	1.32 ± 0.164	1.29 ± 0.045	3			
4. Weybridge	—	—	0.15 <sup>1</sup> , 0.45 <sup>1</sup> , 5.00 <sup>1</sup>	1.19 ± 0.095	1.30 ± 0.126	1.16 ± 0.071	1.13 ± 0.055	1.24 ± 0.035	1.20 ± 0.028	6			
<i>Phaenicia sericata</i>													
1. Montreal	1.65 <sup>1</sup> ± 0.212	1.06 <sup>1</sup> , 1.87 <sup>1</sup> , 7.55 <sup>1</sup>		1.24 ± 0.077	1.61 ± 0.197	1.63 ± 0.152	1.26 ± 0.084	1.37 ± 0.164	1.41 ± 0.045	5			
2. St. Hilaire	2.26 <sup>1</sup> ± 0.195	0.38 <sup>1</sup> , 1.23 <sup>1</sup> , 7.34 <sup>1</sup>		1.27 ± 0.173	1.66 ± 0.126	1.84 ± 0.205	1.23 ± 0.118	1.25 ± 0.141	1.45 ± 0.077	7			
3. Weybridge	1.43 <sup>1</sup>	—	0.40 <sup>1</sup> , 1.09 <sup>1</sup> , 1.66 <sup>1</sup> , 6.68 <sup>1</sup>	1.24 ± 0.870	1.59 ± 0.263	1.58 ± 0.213	1.21 ± 0.497	1.29 ± 0.220	1.38 ± 0.095	6			
Percentages of TCL													
<i>Lucilia illuistris</i>													
1. Algonquin Park	9.4 <sup>1</sup>	—	16.2 ± 1.695	14.6 ± 0.789	16.0 ± 0.468	16.7 ± 0.734	17.5 ± 0.889	19.0 ± 0.532	76.5 ± 7.037	6			
2. Algonquin Park	7.1 <sup>1</sup> ± 1.189	—	17.9 ± 0.775	13.8 ± 0.954	15.5 ± 0.518	16.4 ± 0.533	17.6 ± 0.476	18.8 ± 1.034	69.3 ± 7.567	8			
3. Montreal	—	—	18.6 ± 0.866	13.7 ± 0.463	15.2 ± 0.494	16.7 ± 0.322	17.2 ± 0.514	18.5 ± 0.322	56.7 ± 1.127	3			
4. Weybridge	5.7 <sup>1</sup> ± 0.597	—	15.9 ± 0.369	15.5 ± 0.292	15.7 ± 0.498	16.9 ± 0.546	17.7 ± 0.383	18.1 ± 0.394	57.4 ± 7.556	6			
<i>Phaenicia sericata</i>													
1. Montreal	—	—	5.3	14.3	17.7	19.4	23.3	30.7	50.7	1			
2. St. Hilaire	11.3 <sup>1</sup> ± 0.141	22.9 ± 1.479		13.3 ± 1.069	14.4 ± 0.652	15.3 ± 0.158	16.4 ± 0.321	17.6 ± 0.404	63.3 ± 8.630	5			
3. Weybridge	10.4 <sup>1</sup> ± 0.853	21.5 ± 1.016		13.3 ± 0.515	14.3 ± 1.023	15.5 ± 1.175	17.0 ± 0.923	18.5 ± 0.472	71.2 ± 9.934	7			
	7.9 <sup>1</sup> ± 1.288	19.9 ± 0.807		13.6 ± 0.438	15.0 ± 0.378	15.6 ± 0.766	17.2 ± 0.747	18.6 ± 0.420	64.0 ± 4.290	6			

NOTE: Superscript figures indicate the number of individual chromosomes involved.

*Phaenicia sericata* (Mg.)

The 12-chromosome somatic complements of larvae from three different collections of this species (Table II) have been drawn and analyzed. Eighteen complements from 10 larvae were analyzed. The sexes were presumed to be 3 ♀:1 ♂ of collection No. 1, 1 ♀:3 ♂ of No. 2, and 1 ♀:1 ♂ of No. 3. The average TCL for each collection is listed in Table IV and the ranges of TCL for the three collections in the order listed are 54.9 to 76.2, 55.8 to 84.5, and 59.9 to 69.7  $\mu$ . An idiogram for the species, constructed from data of collection No. 2, is shown in Fig. 8.

Chromosomes of pair I are acrocentric, heteropycnotic, heterochromatic, and 20 to 23% of the TCL. The Y-chromosome is about half the length of the X-chromosome of pair I and also acrocentric, heteropycnotic, and heterochromatic. Pair II is much smaller with a nearly median kinetochore (average ratio 1.25). The slightly longer pair III has a considerably higher arm ratio of about 1.6. Pair IV differs little from III but pair V has a much lower arm ratio, averaging about 1.2. Pair VI is longer, but still considerably shorter than pair I, and has a slightly higher average arm ratio of about 1.3.

Secondary ratios for pair I are shown in Table IV. They are summarized, for the 25 chromosomes of this pair analyzed, as follows: 0.38 for 6, 1.13 for 23, 1.80 for 5, and 7.18 for 24. Secondary ratios for 8 of the total of 11 Y-chromosomes averaged 2.00. Secondary constrictions were not recorded frequently in most of the autosomes (see Table VII). Clearly, the secondary constriction in the short arm of pair V is useful in identifying chromosomes of that pair, but even using this the assignment of correct pair numbers to the autosomes is sometimes difficult and subject to occasional error.

Differences between complements of different collections are of doubtful significance. The X and Y seem to be somewhat smaller in the Weybridge collection and pair IV has a higher arm ratio in the St. Hilaire collection. The Weybridge collection may be a different race or sibling species.

The X- and Y-chromosomes are clearly heteropycnotic and positively heterochromatic. In metabolic nuclei there are other conspicuous chromatic regions, which suggests that parts of some of the autosomes are also heteropycnotic.

## TRIBE CALLIPHORINI

*Eucalliphora lilaea* (Walk.)

I have seen the 12-chromosome complements of this species in the brain cells of larvae contributed by Professor M. T. James of Pullman, Washington. The metaphase plates seen were not suitable for drawing but several features of the karyotype, including the number of chromosomes, were clear. Pair I is small and in general the autosomes resemble those of *Calliphora vicina*.

*Calliphora viridescens* Desr.

Metz (11) lists this species (prob. = *Calliphora viridescens* R.-D.) among those he studied and states "Likewise the other Muscidae studied (*Muscina stabulans*, *Calliphora viridescens*, *Lucilia sericata* and *Pseudopyrellia* sp.) agree with those already described." Since "those already described" refers to *Calliphora erythrocephala*, *Musca domestica*, and *Phormia regina*, all of which

TABLE V  
Somatic chromosome complements of species in the subfamily Caliphorinae, tribe Caliphorini

Collection	Secondary ratios			Primary arm ratios						Averages II-VI	No. comp. ana- lyzed
	Y	I	II	III	IV	V	VI				
Arm ratios											
<i>Caliphora vicina</i>	0.77 <sup>a</sup> ± 0.010	0.30 <sup>a</sup> , 0.88 <sup>a</sup>	1.24 ± 0.100	2.15 ± 0.212	2.00 ± 0.210	1.18 ± 0.110	1.20 ± 0.118	1.55 ± 0.045	12		
1. Montreal	—	—	1.53 ± 0.155	2.19 ± 0.259	1.94 ± 0.134	1.16 ± 0.089	1.20 ± 0.045	1.61 ± 0.141	3		
2. Montreal	1.02 ± 0.173	0.44 <sup>a</sup> , 0.92 <sup>a</sup>	1.23 ± 0.084	2.11 ± 0.237	1.89 ± 0.227	1.17 ± 0.095	1.26 ± 0.134	1.54 ± 0.077	10		
3. Montreal	1.32 ± 0.279	1.21 <sup>a</sup> ± 0.040	1.25 ± 0.147	2.07 ± 0.264	1.95 ± 0.146	1.23 ± 0.434	1.22 ± 0.122	1.59 ± 0.084	9		
<i>Caliphora somatoria</i>	0.70 <sup>a</sup> ± 0.187	0.32 <sup>a</sup> ± 0.071	1.41 ± 0.190	1.86 ± 0.259	2.21 ± 0.197	1.36 ± 0.235	1.33 ± 0.148	1.63 ± 0.095	12		
1. Algonquin Park	0.70 <sup>a</sup> ± 0.187	0.32 <sup>a</sup> ± 0.071	1.41 ± 0.190	1.86 ± 0.259	2.21 ± 0.197	1.36 ± 0.235	1.33 ± 0.148	1.63 ± 0.095	12		
2. Weybridge	1.28 <sup>a</sup> ± 0.257	1.22 <sup>a</sup> ± 0.032	1.55 ± 0.194	1.53 ± 0.183	2.03 ± 0.169	1.38 ± 0.130	1.33 ± 0.164	1.56 ± 0.063	5		
<i>Cynomyopsis cadaverina</i>	0.751	0.35 <sup>a</sup> , 1.57	1.47 ± 0.195	1.57 ± 0.322	3.66 <sup>a</sup> ± 0.288	1.53 ± 0.089	1.38 ± 0.200	1.92 <sup>a</sup> ± 0.110	5		
1. Algonquin Park	0.711	0.27 <sup>a</sup> , 1.79 <sup>a</sup>	1.31 ± 0.170	1.40 ± 0.200	2.25 ± 0.167	1.44 ± 0.214	1.34 ± 0.130	1.57 ± 0.055	10		
2. Montreal	0.52 <sup>a</sup> ± 0.141	0.21 <sup>a</sup> , 1.69 <sup>a</sup>	1.36 ± 0.100	1.66 ± 0.114	2.35 ± 0.226	1.29 ± 0.044	1.41 ± 0.110	1.61 ± 0.055	5		
3. Pullman	0.61 <sup>a</sup> ± 0.080	0.21 <sup>a</sup> , 2.00 <sup>a</sup>	1.38 ± 0.108	1.61 ± 0.020	2.30 ± 0.261	1.30 ± 0.014	1.43 ± 0.115	1.61 ± 0.155	4		
4. Weybridge	—	—	—	—	—	—	—	—	—		
Percentages of TCL											
<i>Caliphora vicina</i>	3.7 <sup>a</sup> ± 0.095	4.7 ± 0.542	15.2 ± 0.829	16.7 ± 0.460	19.9 ± 1.179	21.1 ± 0.974	22.4 ± 0.699	75.7 ± 13.554	12		
1. Montreal	—	—	15.5 ± 0.851	18.3 ± 0.514	18.5 ± 0.155	20.7 ± 0.881	22.2 ± 1.168	55.9 ± 9.920	3		
2. Montreal	3.6 <sup>a</sup> ± 0.548	4.8 ± 0.488	15.7 ± 0.765	16.7 ± 0.519	19.9 ± 0.791	20.6 ± 0.881	22.3 ± 0.644	81.5 ± 3.533	10		
3. Montreal	3.4 <sup>a</sup> ± 0.471	4.5 ± 0.572	16.7 ± 1.656	17.6 ± 1.261	18.5 ± 1.196	20.4 ± 0.851	22.1 ± 1.134	81.8 ± 16.111	9		
<i>Caliphora somatoria</i>	3.4 <sup>a</sup> ± 0.579	5.3 ± 0.775	16.2 ± 0.594	17.8 ± 0.959	18.0 ± 1.243	19.4 ± 1.174	23.3 ± 1.558	75.8 ± 13.395	12		
1. Algonquin Park	3.4 <sup>a</sup> ± 0.579	5.3 ± 0.775	16.2 ± 0.594	17.8 ± 0.959	18.0 ± 1.243	19.4 ± 1.174	23.3 ± 1.558	75.8 ± 13.395	12		
2. Weybridge	3.8 <sup>a</sup> ± 0.458	5.3 ± 0.240	16.3 ± 0.626	18.8 ± 0.537	18.3 ± 0.464	19.4 ± 0.572	22.0 ± 0.847	54.8 ± 6.053	5		
<i>Cynomyopsis cadaverina</i>	2.5 <sup>a</sup> ± 1.012	5.8 ± 1.608	16.2 ± 0.497	17.8 ± 0.673	18.4 ± 0.764	18.9 ± 0.513	22.5 ± 1.863	79.4 ± 20.958	5		
1. Algonquin Park	2.5 <sup>a</sup> ± 1.012	5.8 ± 1.608	16.2 ± 0.497	17.8 ± 0.673	18.4 ± 0.764	18.9 ± 0.513	22.5 ± 1.863	79.4 ± 20.958	5		
2. Montreal	3.8 <sup>a</sup> ± 0.604	6.6 ± 0.092	17.0 ± 0.238	17.8 ± 0.158	18.3 ± 0.476	18.4 ± 0.518	22.0 ± 0.785	84.0 ± 9.426	10		
3. Pullman	3.6 <sup>a</sup> ± 0.404	6.2 ± 0.569	17.0 ± 0.141	17.7 ± 0.153	18.2 ± 0.208	18.2 ± 0.311	22.0 ± 0.686	84.5 ± 3.051	5		
4. Weybridge	3.6 <sup>a</sup> ± 0.404	6.2 ± 0.569	17.0 ± 0.141	17.7 ± 0.153	18.2 ± 0.208	18.2 ± 0.311	22.0 ± 0.686	84.5 ± 3.051	4		

Note: Superscript figures indicate the number of individual chromosomes involved.

<sup>a</sup>Ratio calculated on a different basis, see text.

have six pairs of chromosomes, it seems clear that *C. viridescens* also has six pairs, in general resembling those of the three other species last mentioned.

*Calliphora vicina* R.-D.

Thirty-four chromosome complements, each consisting of 12 chromosomes and coming from larvae of four different collections, have been analyzed (see Table II). The Weybridge collection was labelled *Calliphora erythrocephala*, a synonym as mentioned previously. The presumed sexes of the larvae used for chromosome analysis were: collection No. 1, 2 ♀ and 1 ♂; No. 2, 1 ♀; No. 3, 2 ♀ and 3 ♂; and No. 4, 1 ♀ and 3 ♂. The average TCL for complements of each collection are listed in Table V. Their ranges in length were 52.0 to 90.0  $\mu$  for No. 1, 53.2 to 60.4  $\mu$  for No. 2, 69.5 to 106.9  $\mu$  for No. 3, and 60.6 to 106.6  $\mu$  for No. 4. Results of analysis have also been recorded in Table V and an idiogram for the species, based on results for collection No. 1, is shown in Fig. 9.

The small acrocentric chromosomes of pair I are heteropycnotic and constitute just under 5% of the TCL. The Y-chromosome is also acrocentric, probably heteropycnotic, and corresponds to about 3.5% of the TCL. Pair II is considerably longer than I and has a low arm ratio, about 1.25, except for collection No. 2 in which it is apparently abnormally high in one cell. Pair III has a much higher arm ratio than pair II. Its short arm has two distinct secondary constrictions that serve to distinguish members of this pair from those of pair IV, which are only a little lower in arm ratio. Pairs V and VI have low arm ratios, around 1.2, and VI is noticeably longer than V. Thus the chromosome pairs can be sorted out with considerable accuracy.

Pair I has two secondary constrictions which divide the chromosomes to produce the secondary ratios listed in Table V. Secondary ratios for the Y-chromosomes are also listed in that table. Secondary ratios for the autosomes out of a total of 68 for each pair are shown in Table VII. The secondary constrictions in the long and short arms of pair III and in the long arms of pair IV are the most useful for purposes of identification.

A comparison of the results of analysis for the different collections does not reveal any striking differences. Percentages of the TCL are similar in most cases and differences are of doubtful statistical significance. Primary arm ratios are quite comparable but secondary ratios seem to differ somewhat in the X- and Y-chromosomes for the different collections. The unusually high primary arm ratio for pair II of collection No. 2 is probably the result of a drawing error in one cell. Other differences between primary arm ratios of different collections are probably not significant. Thus in general the differences between collections are of doubtful significance.

*Calliphora vomitoria* (L.)

Collections from Algonquin Park (No. 1) and Weybridge (No. 2) were used for chromosome analysis of this species (see Table II). The 17 somatic complements regularly contained 12 chromosomes and came from 1 ♀ and 5 ♂ of collection No. 1, and 1 ♀ and 2 ♂ of No. 2, presuming that males possess the heteromorphic pair of chromosomes. The TCL averaged 75.8  $\mu$  for collection No. 1 and ranged from 49.7 to 106.5  $\mu$ ; for collection No. 2 the average was 54.8  $\mu$  and the range from 47.7 to 62.6  $\mu$ . Results of analysis have been listed

in Table V; and an idiogram for the species, based on results from collection No. 1, is shown in Fig. 10 and a photomicrograph of the karyotype in Fig. 16.

Pair I chromosomes are acrocentric and heteropycnotic and constitute just over 5% of the TCL. The Y-chromosome is also acrocentric and heteropycnotic and corresponds to about 3.6% of the TCL. Pair II chromosomes are much longer and arm ratios average about 1.5. Pairs III and IV are close to the same length, one being longer in collection No. 1 and the other in collection No. 2. The chromosomes with two clearly distinguishable secondary constrictions in the short arm have been assigned to pair IV. Pairs V and VI have much lower arm ratios than pair IV, and VI is considerably longer than V. Thus the only real problem in analysis concerns which chromosome pair should be called pair III and which pair IV.

Secondary ratios for pair I and the Y-chromosome are shown in Table V. Secondary ratios for autosomal pairs are recorded in Table VII. Those of IVS are of obvious value in identification of chromosomes, and others in IIS, IIL, IIIL, and VIS are of some value in this connection.

The major difference between the results for the two collections has been mentioned above. Also, pair III has a much lower arm ratio in the Weybridge collection. There appear to be differences as well in the positions of secondary constrictions in the X- and Y-chromosomes but confirmation of these points is needed. Secondary ratios of the autosomes were similar in location in the two collections.

#### *Cynomyopsis cadaverina* (R.-D.)

Collections from Algonquin Park, Montreal, Pullman, and Weybridge were used for chromosome analyses of this species (see Table II). All 24 complements analyzed contained 12 chromosomes each. Presumed sexes of the larvae used were: in collection No. 1, 1 ♀; in No. 2, 3 ♀ and 1 ♂; and in No. 3, 2 ♂. The complements of the collections averaged in TCL as shown in Table III and ranged from 61.6 to 107.6  $\mu$  for collection No. 1, from 66.5 to 89.7  $\mu$  for No. 2, from 80.8 to 86.9  $\mu$  for No. 3, and from 81.3 to 88.1  $\mu$  for No. 4 (see Table V and Fig. 11).

Chromosomes of pair I are acrocentric, somewhat heteropycnotic, and constitute about 6% of the TCL. The Y-chromosome is also acrocentric, somewhat heteropycnotic, and corresponds to about 3.5% of the TCL. Pair II chromosomes have a ratio of about 1.4 and are considerably larger than those of pair I. Pair III is longer and has a higher arm ratio than pair II. Pair IV is not much longer than III but its short arm has two distinct secondary constrictions which facilitate recognition of its chromosomes. Failure to recognize the proximal segment of the short arm as belonging to the short arm rather than the long one accounts for the unusually high ratio for pair IV in collection No. 1. The arm ratios of pairs V and VI are much lower than of pair IV, and the greater length of pair VI makes it possible to separate chromosomes of pair V from those of pair VI.

Secondary constrictions were not recorded frequently in chromosomes of this species. The secondary ratios were calculated for 40 autosomes of each pair as shown in Table VII. The secondary constrictions in the short arms of pairs IV and VI are the most useful.

TABLE VI  
Somatic chromosome complements, *Pollenia rudis* (Fab.), subfamily Polleniinae, tribe Pollenini

	I	II	III	IV	V	VI
Arm ratios	—	1.62 ± 0.161	3.07 ± 0.228	1.81 ± 0.145	1.57 ± 0.084	1.64 ± 0.138
Percentage of TCL	4.16 ± 0.511	17.2 ± 0.863	18.1 ± 0.891	18.6 ± 0.480	19.3 ± 0.710	22.6 ± 0.898

TABLE VII  
Main secondary ratios in autosomes of species of Calliphoridae

Species	Chromosome arms:										Total chrom. of each pair
	IIS	IIIL	IIIS	IIIL	IVS	IVL	VS	VL	VIS	VIL	
<i>C. hominivorax</i>	1.46-2		0.61-9		0.65-3	1.00-6	0.88-3	1.32-5	3.00-2	1.70-2	18
<i>C. macillaria</i>	1.33-3		1.15-5		1.06-51	0.91-15	0.66-5	1.09-5	2.68-4	0.52-8	36
<i>Phormia regina</i>	0.75-14	0.87-4	0.93-6	0.95-19					1.20-23	1.36-3	66
<i>P. aenea</i>	0.72-15	0.87-2		1.21-2	1.11-7	2.08-2	1.42-8	1.18-4		0.91-6	22
<i>P. autumn</i>	0.75-13	0.99-10		0.22-3	0.91-11		0.93-4		3.40-11	0.91-6	20
<i>P. metallica</i>	0.72-10	1.02-7	1.17-9	0.73-6	1.03-9	1.80-2	1.25-4	1.92-2	3.10-15	3.36-3	20
<i>L. illustris</i>	1.03-4	1.03-7			0.69-16	0.52-13	0.24-4	0.30-4	0.36-4	0.41-9	46
					3.67-5	3.94-4	0.74-20	0.66-4	1.00-14	1.19-7	
							1.69-5	2.12-5	2.11-4	5.81-4	
							4.09-11	6.00-5	4.02-5		
<i>P. sericata</i>	1.19-3	1.04-5	0.92-6	0.93-6		0.57-3	0.90-14	2.41-6	0.89-7		36
<i>C. vicina</i>	2.09-4	0.77-9	0.59-63	0.70-13	1.25-4	0.79-15	1.32-7	0.45-8	1.24-9	0.58-6	68
			2.53-21	1.19-16		2.05-7			1.90-4	3.21-6	
<i>C. vomitoria</i>	1.09-10	0.88-8	0.41-3	0.87-12	0.52-28	1.09-9	3.95-3	0.83-7	1.15-11	0.78-7	34
					2.38-14				4.06-3		
<i>C. cadaverina</i>	0.99-7	1.12-3	1.07-8	1.04-8	0.34-30	0.57-5	0.95-5	2.21-8	1.49-20	1.00-6	40
			2.67-4	3.05-6	2.00-5	1.15-9	4.74-7			2.64-7	
					2.00-5						
<i>P. rudis</i>	0.90-5	0.64-4		0.38-9	0.93-7	0.66-7	0.92-7	0.82-3	0.60-2	0.58-8	26
				0.92-8						1.46-4	

NOTE: Secondary ratios recorded are averages based on the number of arms shown after each ratio.

The similarity of the results for the four different collections from widely separate sources is interesting. It is notable that the Y-chromosome appears to be unusually small in collection No. 1. There are some differences between collections in the locations of secondary constrictions in the autosomes but these are mostly for constrictions less commonly seen. In general, the different collections gave similar results and those from Pullman and Weybridge yielded almost identical results, indicating that the populations tested represent a fairly homogeneous species.

#### SUBFAMILY POLLENIINAE

##### TRIBE POLLENIINI

#### *Pollenia rudis* (Fab.)

This species is an earthworm parasite and efforts to rear larvae from earthworms have so far proved fruitless. It has been possible, however, to obtain somatic mitotic divisions in ovarian tissue of two adult female flies captured at Macdonald College by Dr. W. F. Grant.

The thirteen 12-chromosome complements analyzed ranged from 49.0 to 68.5  $\mu$  in TCL and averaged 57.6  $\mu$  (though larger complements were seen but not analyzed). Data derived from these analyses have been summarized in Table VI; Fig. 12 shows an idiogram and Fig. 17 a photomicrograph of the karyotype.

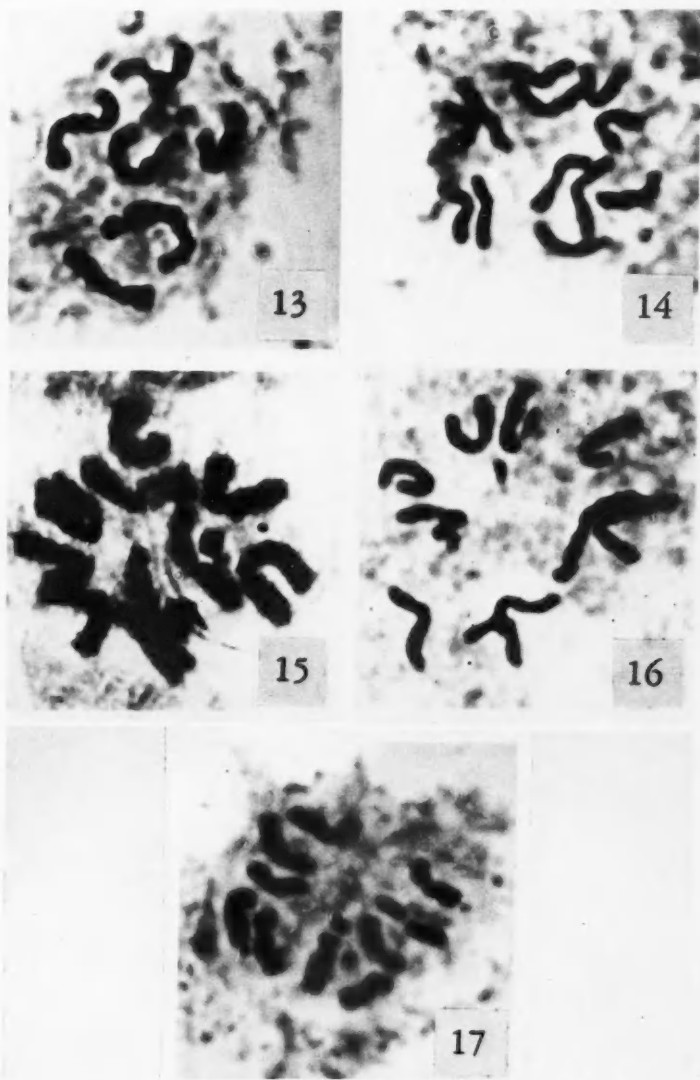
The chromosomes of pair I are acrocentric, and constitute just over 4% of the TCL. Pairs II to VI averaged  $1.95 \pm 0.226$  in arm ratio. Pair II chromosomes are much longer and have an average arm ratio of 1.62. Pair III chromosomes are not much longer but they have a much higher arm ratio, averaging 3.07, which is also much higher than the average of 1.81 for pair IV. Pairs V and VI have lower and more nearly equal arm ratios than pair IV but pair VI is noticeably longer than V. Thus the members of the pairs can be separated in most cases using these two characters.

Secondary constrictions were recorded for the 26 autosomes of each pair and the secondary ratios recorded for IIS in Table VII. The constrictions listed are sometimes useful in identifying chromosomes of the different pairs.

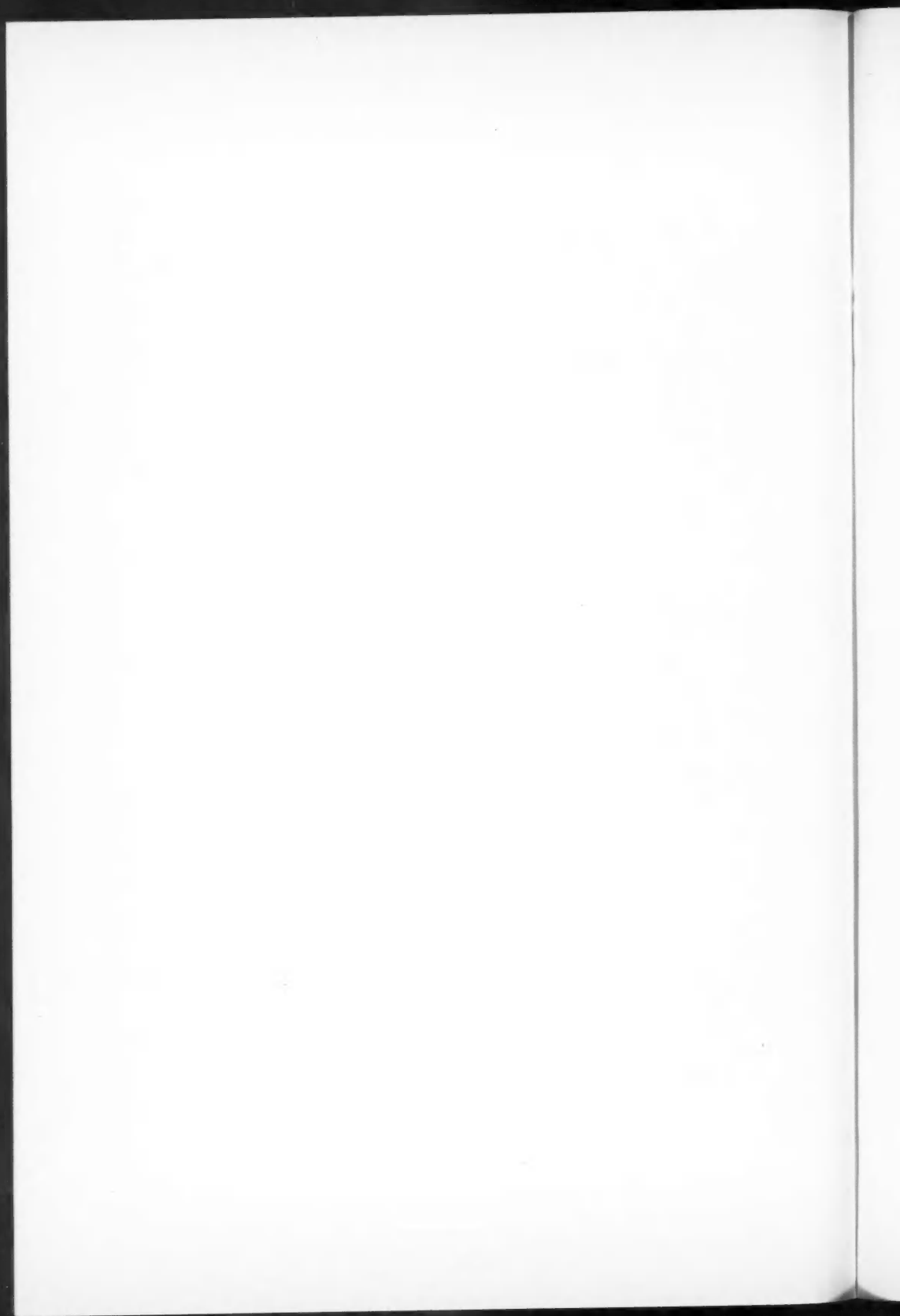
#### Discussion and Conclusion

In *Phormia regina* the results for the three collections are very similar, as might be expected for three samples of the same species from a particular region. In both *Lucilia illustris* and *Phaenicia sericata* the Weybridge collections differ from other collections in pair I and the Y-chromosome enough to suggest that these collections may represent different races or sibling species. These observations suggest that it might be worth while for taxonomists to re-examine collections of *Lucilia illustris* and *Phaenicia sericata* for possible differences between European and North American populations. In *Calliphora vicina*, *Calliphora vomitoria*, and *Cynomyopsis cadaverina* differences between collections are of lower magnitude. Apparently some of these species have progressed further than others in the accumulation of intraspecific chromosomal differences, such as changes in the size relations of the sex chromosomes and the autosomes and in the primary and secondary arm ratios of the autosomes,

PLATE I



FIGS. 13-17. Somatic 12-chromosome complements of Calliphoridae,  $\times 2250$ . Fig. 13. *Lucilia illustris* female, late prophase in larval brain cell, six pairs. Note the intimate pairing of the homologues. Fig. 14. *Lucilia illustris*, female, metaphase in larval brain cell. Fig. 15. *Protocalliphora hirundo*, male, metaphase in spermatogonial cell. Fig. 16. *Calliphora vomitoria*, male, metaphase in spermatogonial cell, X and Y small and centrally located. Fig. 17. *Pollenia rudis*, female, metaphase in ovarian cell, two X's small but distinct.



differences characteristic of interspecific chromosomal variations in higher Diptera.

My results for *Callitroga hominivorax* are not in complete agreement with those of Kaufman and Wasserman. Pair I is nearly 4% of the TCL higher than mine in their idiogram and their arm ratio for pair II is 5.15 as compared with 3.11 for my pair II, which is probably the same pair. There are other differences in the arm ratios. It is, of course, possible that their idiogram is based largely on complements from spermatogonial and oogonial tissue, but even so the differences are puzzling. There are differences also between my results for *Calliphora vicina* and the results obtained from my analyses of published illustrations by Metz, Keuneke, and Naville, shown in Table I, for *Calliphora erythrocephala* (a junior synonym for *C. vicina*). However, their illustrations were mostly from sectioned oogonial and spermatogonial tissues stained in Heidenhain's Iron Haematoxylin or other stains, and it is difficult to be sure about the locations of the kinetochores. Some of their results are puzzling in spite of these considerations. For example, Keuneke has pair I nearly 9% of the TCL, and very different data were obtained for arm ratios than appear to hold for Metz and Naville or those shown in Table V of the present report. In general, my observations confirm those of Strasburger (14) for somatic mitosis in this species. Results of analysis of Metz's illustrations of *Phormia regina* chromosomes are similar to mine but the percentages of TCL differ somewhat. My results for *Protophormia terraenovae* are reasonably close to those obtained from my analysis of Naville's illustrations of the chromosomes of *Phormia terraenovae* (a synonym). Perhaps there has been some confusion in the identification of species in some of these cases, possibly the chromosomes in larval brain cells do not have quite the same morphology as in the spermatogonial or oogonial tissues, and possibly methods of fixation change the morphological characters of chromosomes to some extent. These are problems which need more careful analysis with special modern techniques.

The analyses reported in this paper provide an interesting means of comparing species within certain genera. Differences between the karyotypes of *Callitroga hominivorax* and *Callitroga macellaria* are conspicuous and may be of some assistance in distinguishing larvae of these two species. Differences between the karyotypes of *Protocalliphora* species will be discussed in more detail in a separate report but the greater length of pair I in *P. metallica* and the relatively high arm ratios of pairs II, IV, and V of *P. avium* merit special mention. The differences between chromosomes of *Lucilia illustris* and *L. caesar* have been mentioned above. More data on chromosomes of *L. caesar* are needed and it would be interesting to study the karyotype of *Phaenicia cuprina* from Australia, which seems to be closely related to *L. illustris* and is of economic importance. Similar differences exist between the karyotypes of *Phaenicia sericata* and *P. eximia*. These two examples are particularly interesting because of the great differences between the chromosomes of species of the same genus but they need to be confirmed and supported by studies of other closely related species. The two species of *Calliphora* studied have very small sex chromosomes, and pair III seems relatively smaller in *C. vicina* than what seems to be the corresponding pair, pair IV, in *C. vomitoria* and

*Cynomyopsis cadaverina*. The differences between pairs V and VI seem greater in *C. vomitoria* than in *C. cadaverina*. To date, such careful comparisons of the karyotypes of species within the same genus have repeatedly brought out distinct differences.

Certain comparisons are possible between the genera within particular tribes. Three genera, *Phormia*, *Protophormia*, and *Protocalliphora* (*Apaulena*), belong to the tribe Phormiini. The X- and Y-chromosomes of all three are of similar length and, in all three, secondary constrictions are common in pair I. The average of arm ratios of pairs II-VI inclusive is slightly higher in *Protocalliphora* species. The short arms of pair IV in all three genera have nearly median secondary constrictions. The arm ratio of pair III appears to be considerably higher in *Protophormia* than in the other two genera. In the Luciliini the sex chromosomes vary greatly in size in both *Lucilia* and *Phaenicia*, judged on the basis of present information, but *Lucilia* species seem to have lower II-VI average arm ratios than *Phaenicia*. *Calliphora* and *Cynomyopsis* belong to the tribe Calliphorini with pair I shorter in *Calliphora* than in *Cynomyopsis*. It is, of course, impossible to know whether or not these differences will hold when data are available for other genera and species in these tribes.

Some differences appear to exist between tribes and subfamilies. The several secondary constrictions of pair I and the short arm of pair IV may distinguish members of the Phormiini from those of the Chrysomyini in the subfamily Chrysomyinae. The two genera of Luciliini tend to have very large sex chromosomes not found in other tribes studied to date. Also, II-VI average arm ratios tend to be under 1.55 in the Luciliini and above that value in the Calliphorini. In addition, the X- and Y-chromosomes are regularly short in the Calliphorini. In the Polleniini of the subfamily Polleniinae the single species studied has very short pair I chromosomes, only 4.2% of the TCL, but very high II-VI average arm ratios, 1.95. From such observations, of course, only trends can be suggested, which must be tested in future studies.

The karyotypes studied in the Calliphoridae averaged between 50 and 80  $\mu$  in TCL except in the *Callitroga* species where they averaged about 40  $\mu$  in those complements drawn, perhaps because larger figures in preparations of these species had faded too much to be suitable for drawing. Thus the complements do not seem to differ much in length from those of other families of higher Diptera such as Tachinidae (5), Sarcophagidae (1), and Anthomyiidae (2). It is most interesting that, although many species have small X- and Y-chromosomes of about the same size as in the Anthomyiidae, two species have developed very long and strongly heteropycnotic X- and Y-chromosomes that remind one of those of certain species of Sarcophagidae, such as *Acridrophaga aculeata*, and of species of Tachinidae, such as *Lydella grisescens* and *Winthemia datanae*. It would be most interesting to find out the cause of this parallel tendency to develop large heteropycnotic sex chromosomes in these three families.

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larvae of several calliphorids. Professor Yonge of the Department of Zoology in Glasgow and Professor W. K. Mather of the University of Birmingham kindly permitted me to use facilities in their laboratories while I studied these and other collections. Part of the expense of the collection trip to Europe in 1953 was covered by a grant from the Committee on Research of McGill University. Professor M. T. James and Dr. A. S. Kamal of the Department of Entomology, State College of Washington, sent me fixed larval brains of a number of species. Dr. Gordon F. Bennett of the Ontario Research Foundation contributed many identified larvae of *Protocalliphora* from his personal collections. Professor Wilson Stone of the University of Texas made the arrangements for a grant kindly provided by the National Science Foundation for my collection trip to the Caribbean in 1957. His colleague, Professor Marshall R. Wheeler, helped in many ways. Drs. Gail Kaufman and M. Wasserman contributed larvae of *Callitroga hominivorax*, and Dr. William B. Heed, who accompanied me on the trip, contributed a great deal of time and effort to the success of my work. Part of the cost of my trips to Algonquin Park has been defrayed by a grant from the National Research Council of Canada, and my visits there made pleasant by the kindness of Dr. A. M. Fallis and the capable co-operation of Dr. Gordon F. Bennett, Dr. Roy Anderson, and members of the staff of the Wildlife Research Station. I am also indebted to Dr. Curtis W. Sabrosky of the Division of Insects, U.S. National Museum, Washington, who helped me to collect some of the calliphorids at Algonquin Park and identified them. Dr. Guy E. Shewell of the Entomology Research Institute, Research Branch, of the Canada Department of Agriculture in Ottawa has kindly identified many of the adult flies, a service without which this study could not have been done. Considerable assistance has been given also by my colleague, Professor E. Roger Boothroyd, who drew karyotypes of some of the Caribbean species, and by Miss Katalin Szigeti, who helped with the statistical analysis and drawing the idiograms. To all these generous and helpful persons and also to those institutions and organizations that have contributed financial and other much-appreciated assistance I extend sincere thanks.

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## NOTE

## A METHOD FOR SEPARATING AMOEBIC CYSTS FROM MIXTURES OF TROPHOZOITES AND CYSTS IN CULTURES

E. MEEROVITCH AND BRO. P. LACHANCE

Experimental work with cyst-producing protozoa often requires the separation of cysts from trophozoites for the purposes of counting, measuring, cloning, etc. Trophozoites of parasitic amoebae can be destroyed by treatment with dilute hydrochloric acid and, in the case of *Entamoeba histolytica*, also by chilling (4, 5, 6). In our work with several species of *Entamoeba* (*E. invadens*, *E. terrapinae*, and *E. moshkovskii*), the trophozoites of which are not destroyed by chilling, we found that treatment of culture material with a trichomonacide, marketed under the trade name of "Vagisec" (Julius Schmid (Canada) Ltd., Toronto, Ontario), destroys the trophozoites almost instantaneously, leaving the cysts intact and viable.

"Vagisec" (Carlendacide) is a balanced blend of polyoxyethylene nonyl-phenol, sodium ethylenediamine-tetraacetate, and sodium diocetylsulphosuccinate and was developed for clinical use against *Trichomonas vaginalis* (1, 2, 3). According to the trade literature the ingredients of the preparation, chelating and surface-acting agents, act synergistically to weaken the protozoan cell membrane, to remove waxes and lipids, and to denature the proteins. With the cell membrane destroyed, the organisms swell and explode.

We prepare a 1:100 dilution of "Vagisec" liquid douche concentrate in saline or in water and sterilize by autoclaving. Cultures containing trophozoites and cysts are centrifuged, the supernatant is pipetted off, and the precipitate is mixed with a few milliliters of the diluted "Vagisec". The tube is recentrifuged immediately, and after the centrifuge is brought to a stop in 2-3 minutes, only the cysts remain. If these cysts are to be cultured, "Vagisec" must be removed by two or three washings with saline, water, or the culture medium. In our experience all the cysts remain viable after this treatment and even after an exposure of at least 24 hours to this dilution of "Vagisec".

For purposes of measurement or microscopical study, when it is not desirable to terminate a culture, a drop of culture material containing cysts and trophozoites is placed on a slide, mixed with a drop or two of the diluted "Vagisec", and covered.

A 1:1000 dilution of "Vagisec" takes approximately one minute to destroy the trophozoites of *E. invadens*; dilutions of 1:2000, 1:4000, 1:5000, and 1:7000 take approximately two, three, five, and ten minutes, respectively, while trophozoites remain alive and active for at least thirty minutes in a dilution of 1:10,000.

We find that treatment of cultures of *Entamoeba* with "Vagisec" in order to destroy the trophozoites is more rapid than with hydrochloric acid and less

likely to cause morphological or physiological damage in the cysts. Moreover, it destroys the rounded precystic forms which may sometimes be mistaken for cysts in unstained preparations.

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